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IN VITRO MICROBIOLOGICAL MUTAGENICITY AND UNSCHEDULED DNA SYNTHESIS STUDIES OF FIFTEEN PESTICIDES

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16. ABSTRACT

Fifteen pesticides being reviewed as part of the EPA Substitute Chemical Program were examined by SRI International by several in vitro test procedures, for the following:

- Reverse mutation in Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100 and in Escherichia coli WP2.
- Induction of mitotic recombination in the yeast Saccharomyces cerevisiae D3.
- Relative toxicity in DNA repair-proficient and repair-deficient strains of E. coli (strains W3110 and p3478, respectively) and of Bacillus subtilis (strains H17 and M45, respectively).
- Unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells).

None of the fifteen pesticides demonstrated genetic activity in all six of the in vitro assays. Bioallethrin was the only pesticide that was mutagenic in the S. typhimurium reverse mutation assay. Manzate-D and manzate 200 increased both mitotic recombination in S. cervisiae D3 and UDS in WI-38 cells. Dithane M-22, dithane M-45, ethylchrysanthemate, and zineb increased mitotic recombination in S. cervisiae D3. DL-cis/trans chrysanthemic acid was genotoxic in the relative toxicity assay, being more toxic to the repair-deficient (rec^-) B. subtilis strain M45 than to the repair-proficient (rec^+) strain H17.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH conducts a coordinated environmental health research program in toxicology, epidemiology and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides, and non-ionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

F.Gordon Hueter,Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

Fifteen pesticides being reviewed as part of the EPA Substitute Chemical Program were examined by SRI International by several *in vitro* test procedures, for the following:

- Reverse mutation in *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and in *Escherichia coli* WP2.
- Induction of mitotic recombination in the yeast *Saccharomyces cerevisiae* D3.
- Relative toxicity in DNA repair-proficient and repair-deficient strains of *E. coli* (strains W3110 and p3478, respectively) and of *Bacillus subtilis* (strains H17 and M45, respectively).
- Unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells).

None of the fifteen pesticides demonstrated genetic activity in all six of the *in vitro* assays. Bioallethrin was the only pesticide that was mutagenic in the *S. typhimurium* reverse mutation assay. Manzate-D and manzate 200 increased both mitotic recombination in *S. cerevisiae* D3 and UDS in WI-38 cells. Dithane M-22, dithane M-45, ethylchrysanthemate, and zineb increased mitotic recombination in *S. cerevisiae* D3. DL-*cis*/*trans* chrysanthemic acid was genotoxic in the relative toxicity assay, being more toxic to the repair-deficient (*rec*$^-$) *B. subtilis* strain M45 than to the repair-proficient (*rec*$^+$) strain H17.

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INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act designates the Environmental Protection Agency as the governmental body responsible for the safety of all pesticides used in the United States. More recently, the Federal Environmental Pesticide Control Act (PL 92-516) strengthened EPA's regulatory responsibilities in the area of pesticides to include intra- as well as interstate commerce.

To be federally registered, a pesticide must have been determined not to be hazardous to health or to the environment when used according to its labeling restrictions. Thus, in accordance with new law as well as with specific directives included in Public Law 93-135, 1973, EPA is now conducting a thorough review of the implications of using alternative chemicals, including older registered pesticides, for pest control.

In the pesticide review process, EPA emphasizes development of scientific criteria for evaluating the safety of compounds substituted for those pesticides found to be hazardous. In addition to review and evaluation of the literature on pesticides and maintenance of liaison with industry and academia, the strategy program includes laboratory studies to obtain additional data. One of these laboratory programs is directed toward gathering mutagenesis data on a selected number of compounds.

EPA's program is responsive to one of the recommendations included in the President's Scientific Advisory Committee Report of September 1973, Chemicals and Health. In that document, the Committee recommended that "Regulatory agencies should take steps to insure that new scientific data raising the possibility of new or extended hazards from chemicals in use are subject to careful process of scientific review for merit interpretation".

Development of methods for evaluating the mutagenic hazard of chemical compounds has advanced markedly in the last few years. In contrast to the undefined empirical tests used a short time ago, procedures now available can detect chromosome breaks, DNA damage, and mutational events caused by chemical stress. Mutant strains of microorganisms and human fibroblast cells are effective *in vitro* systems for reliable detection of genotoxic agents.

Many pesticide chemicals in commercial use today have not been adequately investigated for their potential mutagenic hazard. In view of the public's increasing concern about possible pollution of our environment by chemicals, the widely used pesticides must be evaluated.

Under contract to EPA, SRI studied 15 pesticides to determine their potential for mutagenic activity. We used *in vitro* test methods that are appropriate for such evaluations. The 15 pesticides tested are listed in Table 1; the common or trade name, manufacturer, purity, batch or lot number, and supplier are shown. (SRI had previously reported on "*In Vivo* and *In Vitro* Studies of Selected Pesticides to Evaluate Their Potential as Chemical Mutagens" in February 1977[1] and "*In Vitro* Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Eighteen Pesticides" in August 1978.[2] Those studies of 38 pesticides were a part of this contract, No. 68-01-2548, and bring the total number of pesticides tested to 53.)

The six *in vitro* assay systems used were reverse mutation in *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and in *Escherichia coli* WP2; induction of mitotic recombination in the yeast *Saccharomyces cerevisiae* D3; relative toxicity assays in DNA repair-proficient and repair-deficient strains of *E. coli* (strains W3110 and p3478, respectively) and of *Bacillus subtilis* (strains H17 and M45, respectively); and unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells).

METHODS

Microbiological Assays

Fifteen EPA pesticides were examined for genetic activity in five microbiological assay systems: (1) reverse mutation in four strains of histidine-dependent Salmonella typhimurium (TA1535, TA1537, TA98, and TA100); (2) reverse mutation in tryptophan-dependent Escherichia coli WP2; (3) mitotic recombination in Saccharomyces cerevisiae D3; (4) relative toxicity to DNA repair-deficient and DNA repair-proficient strains of Bacillus subtilis (M45 and H17, respectively); and (5) relative toxicity to DNA repair-deficient and DNA repair-proficient strains of E. coli (p3478 and W3110, respectively). An Aroclor 1254-stimulated rat liver metabolic activation system was included in all but the relative toxicity assays to provide steps that the microbes either are incapable of conducting or do not carry out under the assay conditions. The solvent used in all experiments was DMSO.

The assay procedure with S. typhimurium has proved to be 80 to 90% reliable in detecting carcinogens as mutagens; it has about the same reliability in identifying chemicals that are not carcinogens.[3,4] The assay procedure with S. cerevisiae D3 is about 60% reliable in detecting carcinogens as agents that increase mitotic recombination.[5] The E. coli WP2 and the two relative toxicity assays are three additional methods of detecting mutagens; however, the reliability of these test methods has not been adequately validated yet.[6-8] The combination of these five assay procedures significantly enhances the probability of detecting potentially hazardous chemicals.

Salmonella typhimurium Strains TA1535, TA1537, TA98, and TA100

The Salmonella typhimurium strains used at SRI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these

histidine-dependent cells are grown on minimal medium agar plates containing a trace of histidine, only cells that revert to histidine independence (*his*$^+$) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis. The *his*$^+$ revertants are easily scored as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar, the mutation frequency is increased 2- to 100-fold, usually in a dose-related manner.

We obtained our *S. typhimurium* strains from Dr. Bruce Ames of the University of California at Berkeley.[3,9-14] In addition to having mutations in the histidine operon, all the indicator strains have a mutation (*rfa*) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of the vitamin biotin (*bio*) and in the repair of ultraviolet (uv)-induced DNA damage (*uvrb*). The *rfa* mutation makes the strains more permeable to many large aromatic molecules, thereby increasing the mutagenic effect of these molecules. The *uvrB* mutation causes decreased repair of some types of chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to *his*$^+$ by many mutagens that cause base-pair substitutions. TA100 is derived from TA1535 by the introduction of the resistance transfer factor, plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens.[11] In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin,[15] which is a convenient marker to detect the presence of the plasmid in the cell. We have shown that TA100 can detec mutagens such as benzyl chloride and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF2) that are not detected by TA1535. The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens [e.g., ICI-191, benzo(a)pyrene, aflatoxin B_1, and 7,12-dimethylbenz(a)anthracene]. Strain Ta1537 is reverted by many frameshift mutagens. Strain TA98 is derived from TA1538 by the addition of the plasmid pKM101, which makes it more sensitive to some mutagenic agents.

All indicator strains are kept at 4°C on minimal agar plates supplemented with an excess of biotin and histidine. The plates with the plasmid-carrying strains also contain ampicillin (25 μg/ml) to ensure stable maintenance of the plasmid pKM101. New stock culture plates are made every 4 to 6 weeks from single colony reisolates that have been checked for their genotypic characteristics (*his*, *rfa*, *uvrB*, *bio*) and for the presence of the plasmid. For each experiment, an inoculum from the stock culture plates is grown overnight at 37°C in nutrient broth (Oxoid, CM67). After stationary overnight growth, the cultures are shaken for 3 to 4 hours to ensure optimal growth.

To a sterile 13 x 100 mm test tube placed in a 43°C heating block, we add in the following order:

(1) 2.00 ml of 0.6% agar*
(2) 0.05 ml of indicator organisms
(3) 0.50 ml of metabolic activation mixture (optional)†
(4) 0.05 ml of a solution of the test chemical.

For negative controls, we use steps (1), (2), and (3) (optional) and 0.05 ml of the solvent used for the test chemical. For positive controls, we test each culture by specific mutagens known to revert each strain, using steps (1), (2), (3) (optional), and (4).

This mixture is stirred gently and then poured onto minimal agar plates.‡ After the top agar has set, the plates are incubated at 37°C for 2 or 3 days. The number of *his*$^+$ revertant colonies is counted and recorded.

A positive response in the *Salmonella*/microsome assay is indicated by a reproducible, dose-related increase in the number of revertants in one or more of the tester strains.

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*The 0.6% agar contains 0.05 mM histidine, 0.05 mM biotin, and 0.6% NaCl.

†See page 11.

‡Minimal agar plates consist of, per liter, 15 g of agar, 10 g of glucose, 0.2 g of $MgSO_4 \cdot 7H_2O$, 2 g of citric acid monohydrate, 10 g of K_2HPO_4, and 3.5 g of $NaHNH_3PO_4 \cdot 4H_2O$.

Escherichia coli WP2

The E. coli WP2 (uvrA) strain used at SRI was obtained originally from Dr. D. McCalla.[16] It is a tryptophan auxotroph (trp) by virtue of a base-pair substitution mutation in the tryptophan operon. In addition, WP2 is deficient in the repair of some physically or chemically induced DNA damage (uvrA).[17] This uvrA mutation makes the strain more sensitive to certain mutagens.

A procedure similar to the Ames Salmonella assay is used to measure the reversion of WP2 to tryptophan independence. However, the minimal agar is supplemented with 1.25 g of Oxoid nutrient broth (CM67) per liter to provide each plate with the trace of tryptophan required for enhancement of any mutagenic effect of the test chemical.[17] No additional tryptophan is added to the top agar.

Saccharomyces cerevisiae D3

The yeast S. cerevisiae D3 is a diploid microorganism that is heterozygous for a mutation leading to a defective enzyme in the adenine-metabolizing pathway.[18] When grown on medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event may be increased by incubating the organisms with various mutagens (recombinogenic agents). The recombinogenic activity of a compound or its metabolite is determined from the number of red-pigmented colonies appearing on test plates.[19]

A stock culture of S. cerevisiae D3 is stored at 4°C. For each experiment, broth containing 0.05% $MgSO_4$, 0.15% KH_2PO_4, 0.45% $(NH_4)_2SO_4$, 0.35% peptone, 0.5% yeast extract, and 2% dextrose is inoculated with a loopful of the stock culture and incubated overnight at 30°C with shaking.

The in vitro yeast mitotic recombination assay in suspension is conducted as follows. The overnight culture is centrifuged and the cells are resuspended at a concentration of $\sim 10^8$ cells/ml in 67 mM phosphate buffer (pH 7.4). To a sterile test tube are added:

- 1.00 ml of the resuspended culture
- 0.50 ml of either the metabolic activation mixture or buffer
- 0.20 ml of a solution of pesticide dissolved in DMSO or 0.20 ml of DMSO alone
- 0.30 ml of buffer.

Several doses of the pesticide (up to 5%, w/v or v/v) are tested in each experiment and appropriate controls are included.

The suspension mixture is incubated at 30°C for 4 hours on a roller drum. The sample is diluted serially in sterile physiological saline and 0.2-ml aliquots of the 10^{-5} and 10^{-3} dilutions are spread on tryptone-yeast agar plates; five plates are used for the 10^{-3} dilution, and three plates are used for the 10^{-5} dilution. The plates are incubated for 2 days at 30°C, followed by 2 days at 4°C to enhance the development of the red pigment indicative of adenine-deficient homozygosity. Plates of the 10^{-3} dilution are scanned with a dissecting microscope at 10X magnification and the number of red colonies or red sectors (mitotic recombinants) is recorded. The surviving fraction of organisms is determined from the number of colonies appearing on the plates of the 10^{-5} dilution.

The number of mitotic recombinants is calculated per 10^5 survivors. A positive response in this assay is indicated by a dose-related increase in the absolute number of mitotic recombinants per milliliter as well as in the relative number of mitotic recombinants per 10^5 survivors.

Escherichia coli W3110/p3478
and Bacillus subtilis H17/M45

We obtained E. coli strains W3110 and p3478 from Dr. H. Rosenkranz, who devised a differential toxicity/repair assay.[20] Strain p3478 is a DNA (polA-) derivative of W3110. The enzyme DNA polymerase A is involved in resynthesizing DNA segments that have been damaged and excised.[21] Because strain p3478 lacks DNA polymerase, DNA damage (caused by physical or chemical agents that react with DNA) is not repaired as efficiently as it is in the parent strain, W3110. Exposure to physical or chemical agents that interact with DNA will usually inhibit the

growth of p3478 more than that of W3110. Therefore, test compounds that are more toxic to p3478 than to W3110 are probably reacting with the DNA. Test compounds that do not react with DNA are generally equally toxic (or nontoxic) to both strains. The ability of a test compound to induce DNA damage can be evaluated by comparing its relative toxicity (zone of inhibition of growth) to these two strains.

B. subtilis strains H17 and M45 were obtained from Dr. T. Kada.[22] Strain M45 is a recombination-deficient (rec-) derivative of H17. Recombination is required for repair of some types of damaged DNA. Strain M45 lacks the rec function, so damage caused by agents that react with DNA is not as efficiently repaired in M45 as it is in the parent strain, H17. As in the E. coli W3110/p3478 repair assay, the ability of a test compound to induce DNA damage can be evaluated by comparing its relative toxicity to the rec^+ (H17) and rec^- (M45) strains.

For each experiment a 1-ml inoculum of a frozen (-80°C) stock culture is grown overnight at 37°C, with shaking, in broth consisting of 0.5% yeast extract, 0.35% peptone, 2% glucose, 0.05% $MgSO_4$, 0.15% KH_2PO_4, and 0.45% $(NH_4)_2SO_4$. To 2 ml of top agar (0.6% NaCl and 0.6% agar) is added 0.1 ml of the overnight bacterial culture (approximately 3×10^8 cells). The suspension is mixed and poured onto the surface of a plate containing the same ingredients as the broth, plus 2% agar (25 ml). When the top agar has solidified, a sterile filter paper disc (6-mm diameter) is placed on the top agar and impregnated with the test substance, Plates are incubated at 37°C for 16 to 17 hours, and then the diameter of the zone of inhibition of growth is measured.

Several concentrations of the test substances are tested in duplicate. Methylmethanesulfonate is used as the positive control in these assays. The negative control is ampicillin, which causes equal zones of inhibition to repair-proficient and repair-deficient strains because it kills the bacteria by interfering with cell wall synthesis, not by interacting with the DNA.

Aroclor 1254-Stimulated Metabolic Activation System

Some carcinogenic chemicals (e.g., of the aromatic amino type or the polycyclic hydrocarbon type) are inactive unless they are metabolized to active forms. In animals and man, an enzyme system in the liver or other organs (e.g., lung or kidney) is capable of metabolizing a large number of these chemicals to carcinogens.[12-14,23] Some of these intermediate metabolites are very potent mutagens in the S. typhimurium test. Ames has described the liver metabolic activation system that we use.[14] In brief, adult male rats (250 to 300 g) are given a single 500-mg/kg intraperitoneal injection of Aroclor 1254. This treatment enhances the synthesis of enzymes involved in the metabolic conversion of chemicals. Four days after the injection, the animals' food is removed but drinking water is provided ad libitum. On the fifth day, the rats are killed and the liver homogenate is prepared as follows.

The livers are removed aseptically and placed in a preweighed sterile glass beaker. The organ weight is determined, and all subsequent operations are conducted in an ice bath. The livers are washed in an equal volume of cold, sterile 0.15 M KCl (1 ml/g of wet organ), minced with sterile surgical scissors in three volumes of 0.15 M KCl, and homogenized with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 x g, and the supernatant, referred to as the S-9 fraction, is quickly frozen in dry ice and stored at -80°C.

The metabolic activation mixture for each experiment consists of, for 10 ml:

- 1.00 ml of S-9 fraction
- 0.20 ml of $MgCl_2$ (0.4 M) and KCl (1.65 M)
- 0.05 ml of glucose-6-phosphate (1 M)
- 0.40 ml of NADP (0.1 M)
- 5.00 ml of sodium phosphate buffer (0.2 M, pH 7.4)
- 3.35 ml of H_2O.

Unscheduled DNA Synthesis Assays

Many mutagenic and carcinogenic agents have been shown to induce unscheduled DNA synthesis (UDS) in an in vitro tissue culture system

of mammalian cells.[24] UDS is a form of mammalian repair synthesis that involves at least two processes: first, the damaged DNA region is enzymatically removed; then follows incorporation of nucleotides to repair the DNA. UDS, which occurs in a wide variety of mammalian cell types, is considered to be a fairly universal system because it has been observed in all stages of the cell cycle (G_0, G_1, G_2, and M) other than S, the normal synthetic phase.[25,26] (UDS is not observed during S-phase because the high level of incorporation of nucleotides during scheduled DNA synthesis obscures the relatively low level of incorporation of nucleotides during UDS.)

A number of chemicals have been shown to be ineffective in producing DNA damage in in vitro cultures of mammalian cells unless an S-9 preparation from a mammalian liver homogenate and appropriate cofactors are added to the test system. Thus, we routinely perform a parallel series of UDS assays in the presence and absence of a metabolically active environment.

We used the UDS assay system in the previous testing of 38 substitute pesticides.[1,2] Reported here are the results of UDS testing, with and without metabolic activation, of 15 pyrethroid pesticides.

Cell Culture

WI-38 cells grown in T-25 tissue culture flasks were used for the UDS assays. Replicate cultures of these cells were initiated in Eagle's Basal Medium containing 10% (v/v) fetal calf serum. The cells were grown to confluency and were maintained in medium containing 0.5% serum for 5 to 6 days preceding the UDS assay.* This produced contact-inhibited cells in synchronous cultures in the G_0 phase of the mitotic cycle. To

* As a check against the presence of mycoplasma, which could incorporate tritiated thymidine (^{3}H-TdR) and thus obscure measurements of UDS, stock cultures were periodically sent to Microbiological Associates, who cultured them on Difco Beef Heart Infusion agar or broth for analysis for the presence of mycoplasma. The results of these analyses were consistently negative.

further reduce the possibility of incorporation of ^{3}H-TdR by an occasional S-phase cell that might escape the contact-inhibition synchrony and thus obscure measurements of UDS, the cultures were preincubated for 1 hour with 10^{-2} M hydroxyurea (HU) before each assay and 10^{-2} M HU was added during each subsequent step of the assays.

Dilution of Compounds

Immediately prior to each assay, the pesticide was diluted in DMSO to form a series of concentrations that, when diluted into culture medium, yielded the appropriate set of test concentrations. To facilitate solubilization or to achieve an even suspension of the stock solutions of the compounds in solvent, some of the compounds were sonicated for a brief period of time prior to dilution. The final concentration of DMSO was maintained at 1% or less, which we have previously found to be not cytotoxic. In defining the concentrations tested, solid compounds are assumed to be volumeless and compounds in the liquid state are assumed to have a density of 1.00.

Metabolic Activation

For testing with metabolic activation, a preparation consisting of the 9000 x g supernatant of a liver homogenate (250 mg of liver/ml) from adult Swiss-Webster mice was used. To this was added the following cofactors: nicotinamide, 3.05 mg/ml; glucose-6-phosphate, 16.1 mg/ml; $MgCl_2 \cdot 6H_2O$, 5.08 mg/ml; and NADP, 0.765 mg/ml.

Controls

The positive controls were 4-nitroquinoline-N-oxide (4NQO), a compound that induces UDS in the absence of a metabolic activation system, and dimethylnitrosamine (DMN), a compound that induces UDS *in vitro* only when an exogenous metabolic activation system is incorporated into the treatment protocol. The negative control was DMSO diluted in culture medium.

Preliminary Testing

Preliminary testing was performed according to the procedure described for UDS assays. However, this testing differed in two respects: (1) a different number of concentrations spanning a much broader dose range was tested; and (2) fewer replicate samples were tested at each concentration. This testing was conducted for the purpose of establishing an appropriate dose range for each UDS assay.

Test Procedure

The contact-inhibited WI-38 cells were incubated at 37°C with dilutions of the pesticides and with 1 μCu/ml of ^{3}H-TdR (specific activity, 6.7 Ci/mmole). For testing in the absence of metabolic activation, the cells were exposed simultaneously to the pesticide and to ^{3}H-TdR for 3 hours. For testing with metabolic activation, the cells were incubated together with pesticide, ^{3}H-TdR, and the metabolic activation preparation for 1 hour. (The shorter exposure time for metabolic activation testing was used because longer exposures of WI-38 cells to the liver homogenate preparation could be cytotoxic.) In both cases, the cells were then incubated with ^{3}H-TdR and HU, but without pesticide, for an additional 3 hours.

DNA was extracted from the cells using a modification of the PCA-hydrolysis procedure;[27] one aliquot of the DNA solution was used to measure the DNA content after the reaction with diphenylamine,[28] and a second aliquot was used for scintillation-counting measurements of the extent of incorporation of ^{3}H-TdR. The results were expressed as disintegrations per minute (dpm) of incorporated ^{3}H-TdR per unit of DNA and were compared with the rate of incorporation of ^{3}H-TdR into cells exposed to solvent only (negative controls).

We have defined as an acceptable assay one in which the response of the positive control compound is predicted, within the 95% confidence limits, by regressions of average dpm/μg DNA versus average dpm/μg for background.[29] The regressions that follow are based on data that we have acquired in previous testing:

Type of Testing	Regression*	Sample Size (n)	Correlation Coefficient
Without metabolic activation	$Y_1 = 629 + 16.42\ (X)$†	55	0.8066
With metabolic activation	$Y_2 = 212 + 2.11\ (X)$†	25	0.8307

If the observed average level of incorporation for the positive control compound is outside the 95% confidence limits of the regression, we assume that some variation has occurred in the experimental procedures and the test is repeated.

Interpretation of Results

We have tested 41 compounds of defined carcinogenic activity, based on the results of *in vivo* bioassays, and have analyzed these results using either the parametric One-Way Analysis of Variance or the non-parametric Kruskal-Wallis One-Way Analysis of Variance, depending on which was more appropriate.‡ Of the 16 compounds generally recognized as being direct-acting carcinogens, 15 induced statistically significant elevations in the incorporation of ^{3}H-TdR into DNA, at the 99% confidence level. In all but three of these, the response was dose-related. The assay of the sixteenth carcinogen, *p*-rosaniline, failed to suggest a positive response. Of the 13 compounds reported to be noncarcinogenic, only one (glycidol) had a statistically significant positive response, which was not dose-related. Thus it appears that the 99% confidence limits of these statistical analysis coupled with the indication of a dose-related relationship can be used with reasonable accuracy to predict the biological significance of the UDS response to

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*Regressions over a range of background dpm/μg DNA of 0 to 450.

†Y_1 = Average dpm/μg DNA for 10^{-3} M 4NQO (positive control).
Y_2 = Average dpm/μg DNA for 5×10^{-2} M DMN (positive control).
X = Average dpm/μg DNA for background (negative control).

‡If there is reason to believe that the variances of each of the treatments in a test are equal (i.e., Bartlett's test of the variance is negative), the parametric analysis is the appropriate one. If the variances are not equal, the nonparametric analysis is the appropriate one.[30,31]

an ultimate carcinogen or a noncarcinogen.

The correlation between UDS response and biological significance for testing with metabolic activation is less clear. Of the 12 procarcinogens (compounds requiring chemical modifications to become active) that we have tested with metabolic activation, seven induced statistically significant increases in ^{3}H-TdR uptake at the 99% confidence level, all of which were dose-related. The remaining five procarcinogens failed to indicate any increase in ^{3}H-TdR incorporation. Thus, it appears that the metabolic activation preparation presently used for UDS testing is capable of activating only a portion of the spectrum of procarcinogens. However, for those that can be activated, a statistically significant and dose-related response can be observed. Thus, if these two criteria are met, we can interpret the results as indicating the repair of DNA damage by the cells in response to the compound being evaluated. However, lack of a positive response in testing cannot be assumed to be indicative of an absence of potential biological hazard.

RESULTS AND DISCUSSION

The results of the *in vitro* microbiological and UDS assays of the 15 compounds are summarized in Table 2. A positive response in these assays is defined as a reproducible dose-related increase in the effect being observed. A genotoxic or mutagenic effect was observed for eight of the 15 pesticides tested. The eight pesticides that had a positive response in one or more of the assays were bioallethrin, DL-*cis*/*trans* chrysanthemic acid, dithane M22, dithane M-45, ethylchrysanthemate, manzate-D, manzate 200 and zineb. None of the 15 pesticides tested were positive in all six of the *in vitro* assays. Bioallethrin was the only compound mutagenic in the *Salmonella*/microsome assay. Dithane M-22, dithane M-45, ethylchrysanthemate, manzate-D, manzate 200, and zineb all increased mitotic recombination in *S. cerevisiae* D3, but only manzate-D and manzate 200 also induced unscheduled DNA synthesis in WI-38 cells. DL-*cis*/*trans* chrysanthemic acid was genotoxic in the *B. subtilis* relative toxicity assay but was inactive in all other assays. Dose-response curves are presented for pesticides that give a positive response in the assays except for the relative toxicity assays. Biphenyl, chlordimeform, NRDC-149, permethrin, polyram, resmethrin, and sumithrin were not genotoxic or mutagenic in any of the six assays we performed.

Microbiological Assays

Each pesticide was tested at least twice on separate days, using one plate per dose. The first experiment was a test over a wide range of doses to look for toxicity or mutagenicity. If no toxicity or mutagenicity was observed, the second experiment was conducted at higher concentrations. If mutagenicity was observed, a dose response was determined. An assay that gave a mutagenic response was always

repeated to confirm that the results were reproducible.

Tables 3 through 37 present the results of the assays using strains TA1535, TA1537, TA98, and TA100 of the bacteria Salmonella typhimurium and Escherichia coli WP2. In the standard Ames Salmonella/microsome assay, bioallethrin was the only pesticide found to be mutagenic. Bioallethrin (Tables 3 and 4) was tested with all strains once over a dose range of 50 to 10,000 µg/plate and retested over a range of 500 to 20,000 µg/plate. A third experiment using only TA100 in the presence of metabolic activation, with doses ranging from 10 to 2500 µg/plate, is presented in Table 5. Bioallethrin appeared to be mutagenic when metabolic activation was provided; without activation, no significant increase in revertants was observed. Figure 1 shows the dose response. No increase in revertants occurred with E. coli WP2.

The results for biphenyl (Tables 6 and 7), chlordimeform (Tables 8 and 9), DL-cis/trans chrysanthemic acid (Tables 10 and 11), dithane M-22 (Tables 12 and 13), dithane M-45 (Tables 14-16), ethylchrysanthemate (Tables 17 and 18), manzate-D (Tables 19 and 20), manzate 200 (Tables 21 and 22), NRDC-149 (Tables 23-25), permethrin (Tables 26 and 27), polyram (Tables 28 and 29), resmithrin (Tables 30 and 31), sumithrin (Tables 32-34), and zineb (Tables 35-37) all failed to demonstrate a reproducible, dose-related increase in the number of revertants with and without metabolic activation.

The results appearing in Tables 38 through 67 represent the findings of the assays for mitotic recombination in Saccharomyces cerevisiae D3. Six pesticides--dithane M-22, dithane M-45, ethylchrysanthemate, manzate-D, manzate 200, and zineb--increased the mitotic recombination frequency in each experiment and are considered positive by these procedures.

DL-cis/trans chrysanthemic acid was tested over a range of 0.5 to 0.9% and retested over a range of 0.6 to 0.8% (Tables 44 and 45).

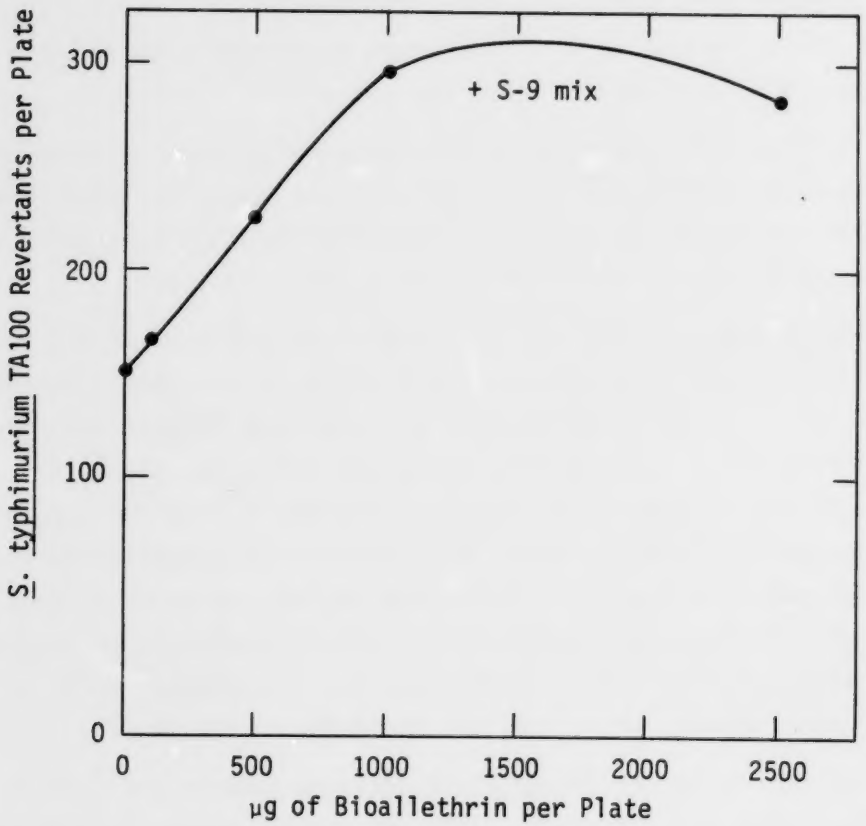

FIGURE 1 ASSAY OF BIOALLETHRIN WITH SALMONELLA TYPHIMURIUM STRAIN TA100 WITH METABOLIC ACTIVATION

This compound demonstrated a narrow range of toxicity--the level of survival was 100% at a concentration of 0.7%, but the compound was toxic at 0.8%. Although some of the values for mitotic recombinant per 10^5 survivors are high without the metabolic activation system, there is no reproducible dose-related increase in the number of recombinants; thus, we conclude that DL-cis/trans chrysanthemic acid is not recombinogenic.

Dithane M-22 increased mitotic recombinants in the absence of metabolic activation at a concentration of 0.005%. Tables 46 and 47 present the data; Figure 2 shows the curve.

Dithane M-45 showed recombinogenic activity both in the presence and absence of metabolic activation. It was tested at a dose range of 0.001 to 0.01%. Toxicity occurred above 0.01%. The results are presented in Tables 48 and 49; Figure 3 shows the curve.

Ethylchrysanthemate was first tested over a range of 0.5 to 4.0% without metabolic activation and of 1 to 4% with metabolic activation (Table 50). It was retested over the same range without metabolic activation and at 1 to 5% with activation (Table 51; Figure 4). Although recombinogenic activity was observed in both the presence and absence of metabolic activation, addition of metabolic activity reduced recombinogenicity. A possible explanation of this event is that the metabolic activation system acts to metabolize the compound to less genetically active metabolites or, by nonspecifically reacting with the compound, makes them less available to the yeast.

Manzate-D (Tables 52 and 53, Figure 5), manzate 200 (Tables 54 and 55, Figure 6), and zineb (Tables 66 and 67; Figure 7) induced increases in the number of mitotic recombinants in both the presence and absence of the metabolic activation system.

Table 68 presents the results of the differential toxicity assessed in DNA repair-proficient and DNA repair-deficient strains of E. coli (W3110 and p3478) and of B. subtilis (H17 and M45) with the

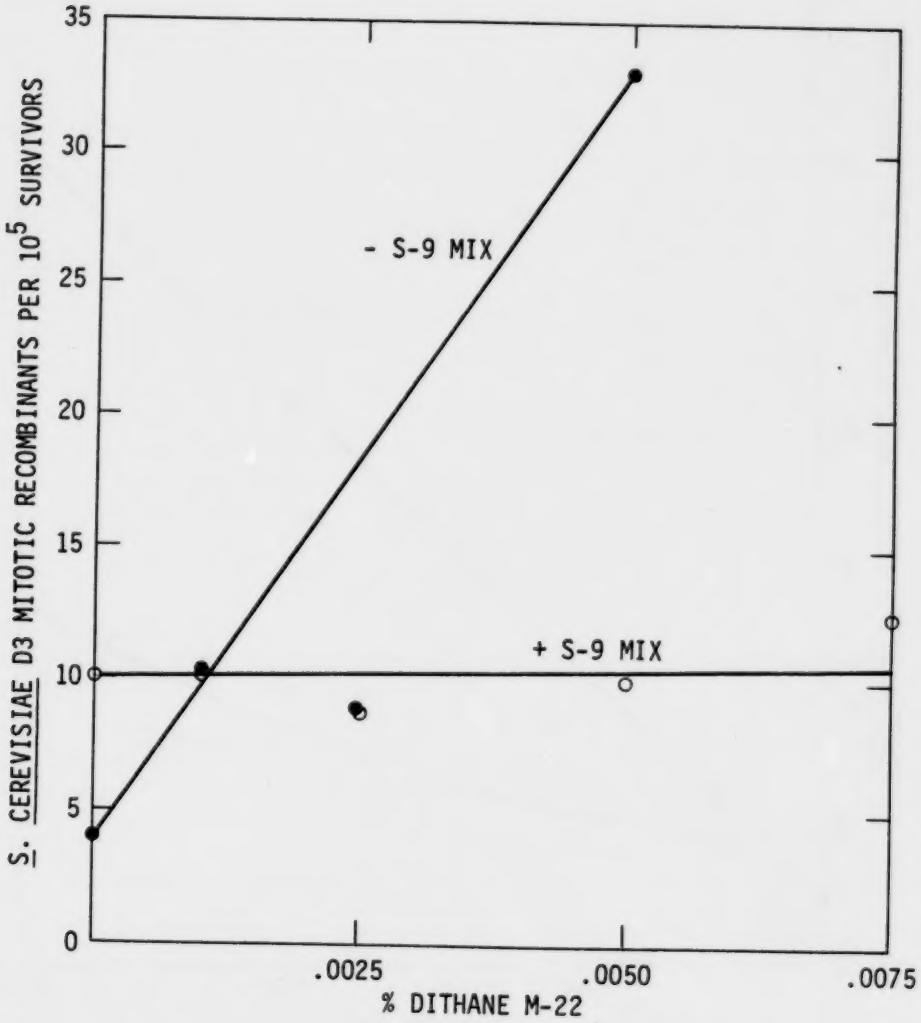

FIGURE 2 ASSAYS OF DITHANE M-22 WITH SACCHAROMYCES CEREVISIAE D3

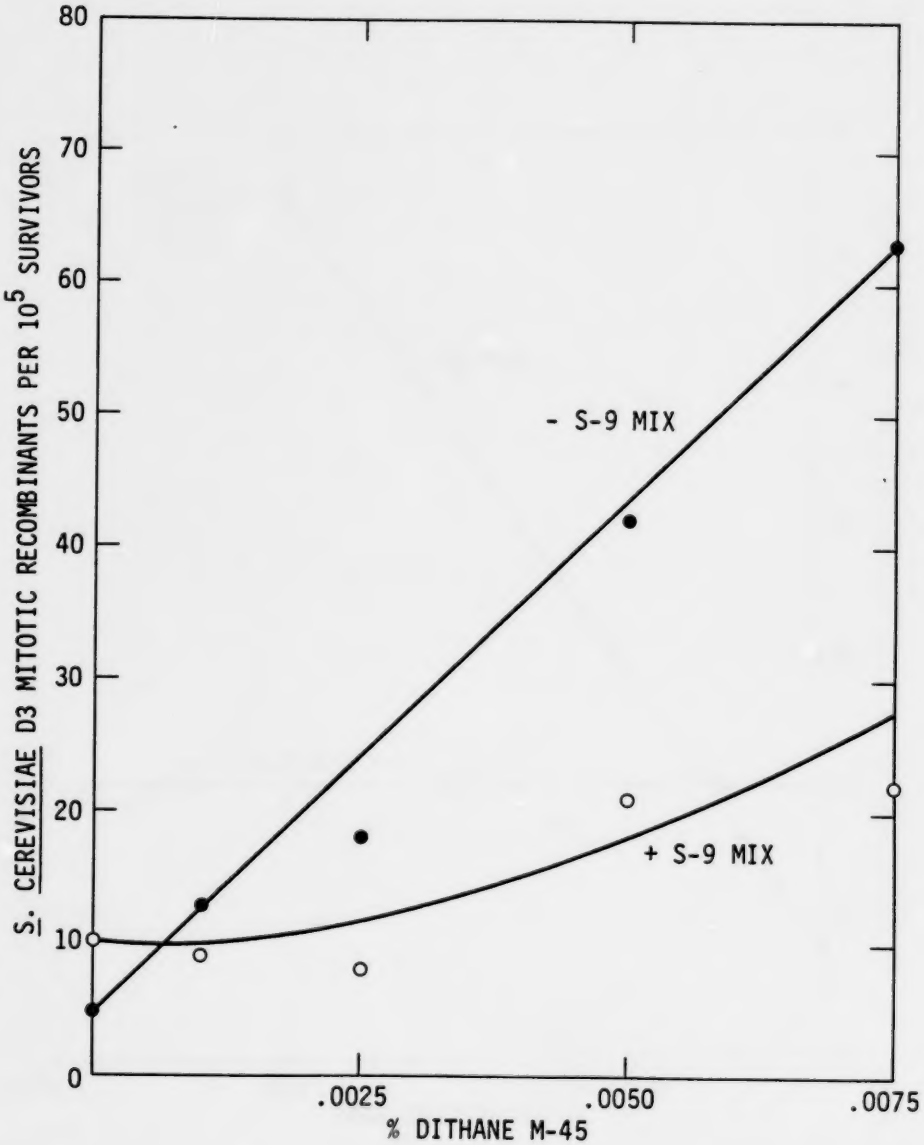

FIGURE 3 ASSAYS OF DITHANE M-45 WITH SACCHAROMYCES CEREVISIAE D3

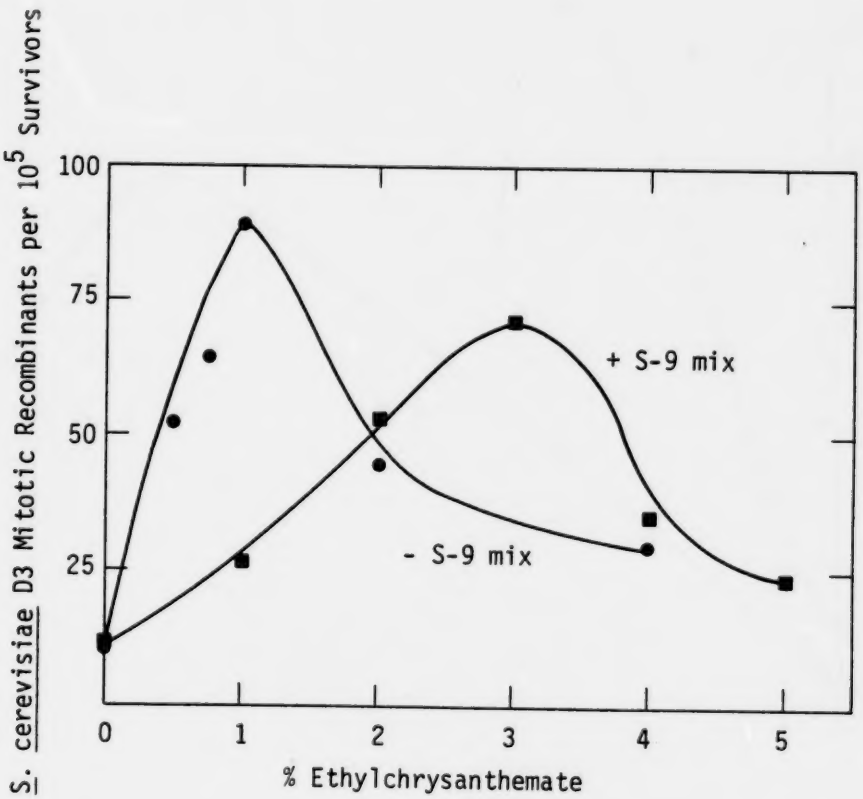

FIGURE 4 ASSAYS OF ETHYLCHRYSANTHEMATE WITH SACCHAROMYCES CEREVISIAE D3

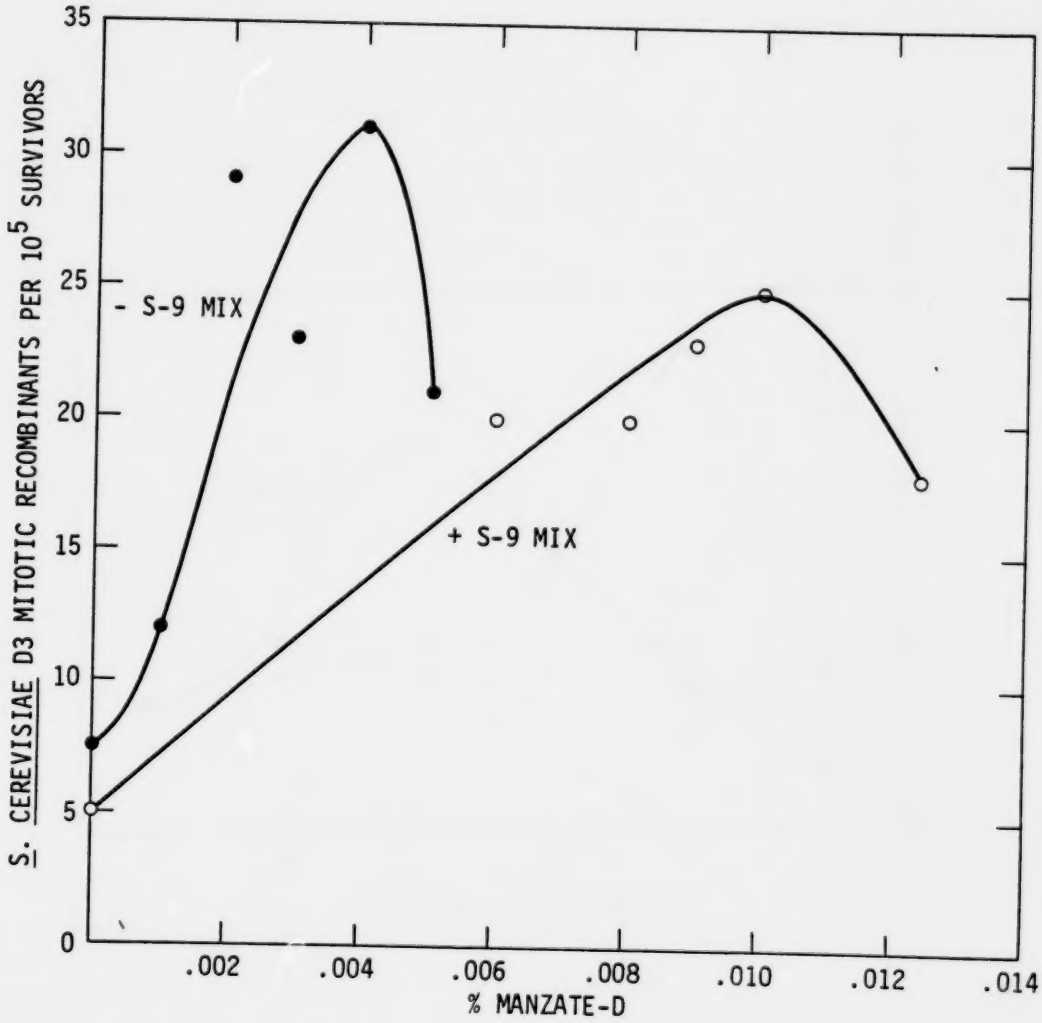

FIGURE 5 ASSAYS OF MANZATE-D WITH SACCHAROMYCES CEREVISIAE D3

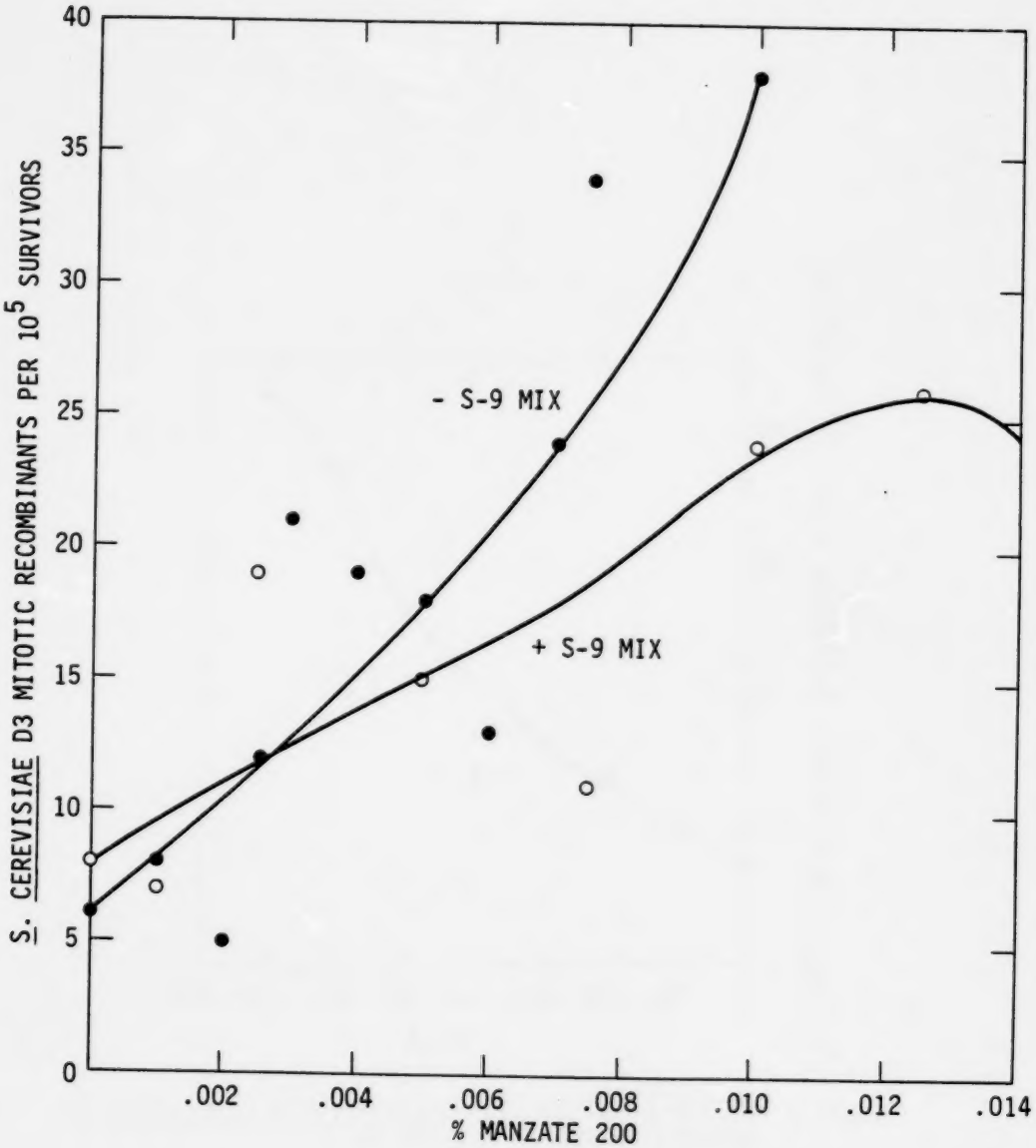

FIGURE 6 ASSAYS OF MANZATE 200 WITH SACCHAROMYCES CEREVISIAE D3

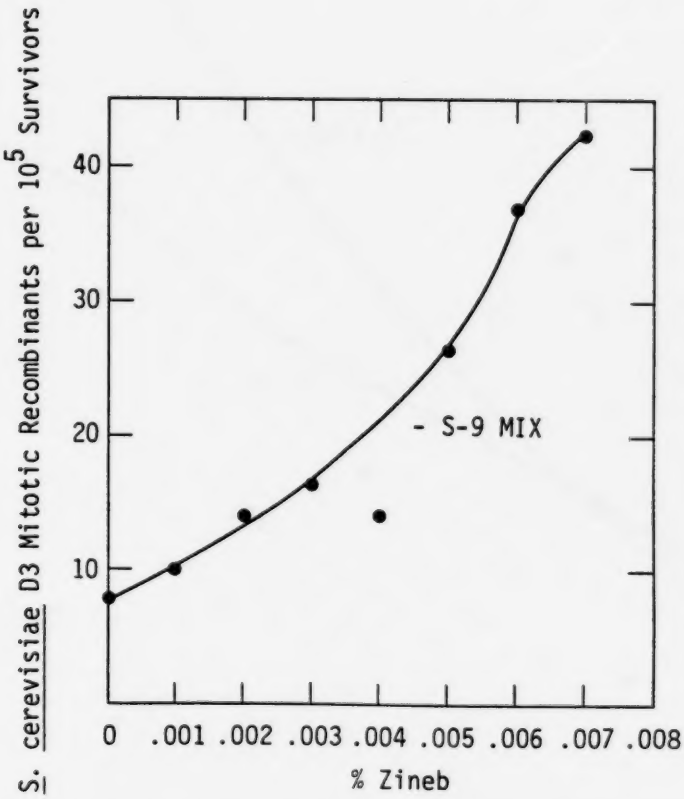

FIGURE 7 ASSAYS OF ZINEB WITH SACCHAROMYCES CEREVISIAE D3 WITHOUT METABOLIC ACTIVATION

15 pesticides. Toxic chemicals that do not act by damaging DNA (e.g., ampicillin) should give equal zones of toxicity on both DNA repair-proficient and DNA repair-deficient strains. However, a given concentration of a chemical that is genotoxic may be more toxic for a repair-deficient strain than for a strain that effectively repairs its DNA.

Results in which no growth inhibition was observed could indicate that either the pesticide did not diffuse into the agar, the pesticide was not able to penetrate the cell, or the concentration of the test dose was not high enough. The differential toxicity assays of E. coli with bioallethrin, chlordimeform, and DL-cis/trans chrysanthemic acid showed no growth inhibition. Biphenyl, NRDC-149, permethrin, resmethrin, and simithrin caused no growth inhibition in either E. coli or B. subtilis. Therefore, the results from these assays remain inconclusive.

Bioallethrin and chlordimeform induced approximately equal zones of inhibition in assays with B. subtilis. Dithane M-22, dithane M-45, ethylchrysanthemate, manzate-D, manzate 200, polyram, and zineb all induced approximately equal zones of inhibition in both E. coli and B. subtilis. The results from these assays are considered negative.

DL-cis/trans chrysanthemic acid caused a slightly larger zone of inhibition, at all doses tested, in the rec^- strain (B. subtilis M45) than in the rec^+ strain (B. subtilis H17).

UDS ASSAYS

The results of the UDS testing of 15 pyrethroid pesticides are presented in Tables 69 through 133. For each compound, preliminary assays were performed with and without metabolic activation to establish the appropriate range of concentrations to be tested in the actual UDS assays. The range of test concentrations to be used for each compound was then selected according to the following criteria: (1) if a positive response was indicated by the preliminary assay,

concentrations that would confirm the response and define a dose-response relationship were tested; (2) in the absence of a preliminary indication of a positive response, concentrations that were expected to cover the range between cytotoxic effects and no effect were tested; and (3) if the preliminary assay did not indicate either a positive response or cytotoxic effects, a range of concentrations near the maximum feasible under the constraints of the solubility of the compound and the protocols described in "Dilution of Compounds" was tested.

In the absence of metabolic activation, the initial testing of bioallethrin (Tables 69 and 70) suggested an increase in UDS. The results presented in Table 70 were statistically positive ($H = 20.16 \simeq \chi^2_5 > 15.09$) but did not clearly indicate a dose-response. Therefore, a second UDS assay was conducted without metabolic activation. The results of this test (Table 71) failed to indicate an increase in ^{3}H-TdR uptake. Therefore, the results of the testing of bioallethrin without metabolic activation must be considered negative. Likewise, the results observed in the testing of this compound with metabolic activation (Tables 72 and 73) failed to suggest an increase in UDS, and are interpreted as being negative.

Tables 98 through 107 present the results of the testing of manzate-D and manzate 200. In each case, the preliminary assays without metabolic activation (Tables 98 and 103) indicated cytotoxic effects at concentrations down to approximately 20 µg/ml, with no effects at 2 µg/ml. The UDS assays were then conducted over a range of concentrations from approximately 1 to 30 µg/ml. The results of these tests (Tables 99 and 104) indicated an elevation in ^{3}H-TdR incorporation at a concentration of 5 µg/ml, but failed to define a dose response. Therefore, a second assay of each without metabolic activation was performed over an even more narrow concentration range. The results of these assays (Tables 100 and 105; Figures 8 and 9) indicated dose-dependent, statistically significant increases in UDS

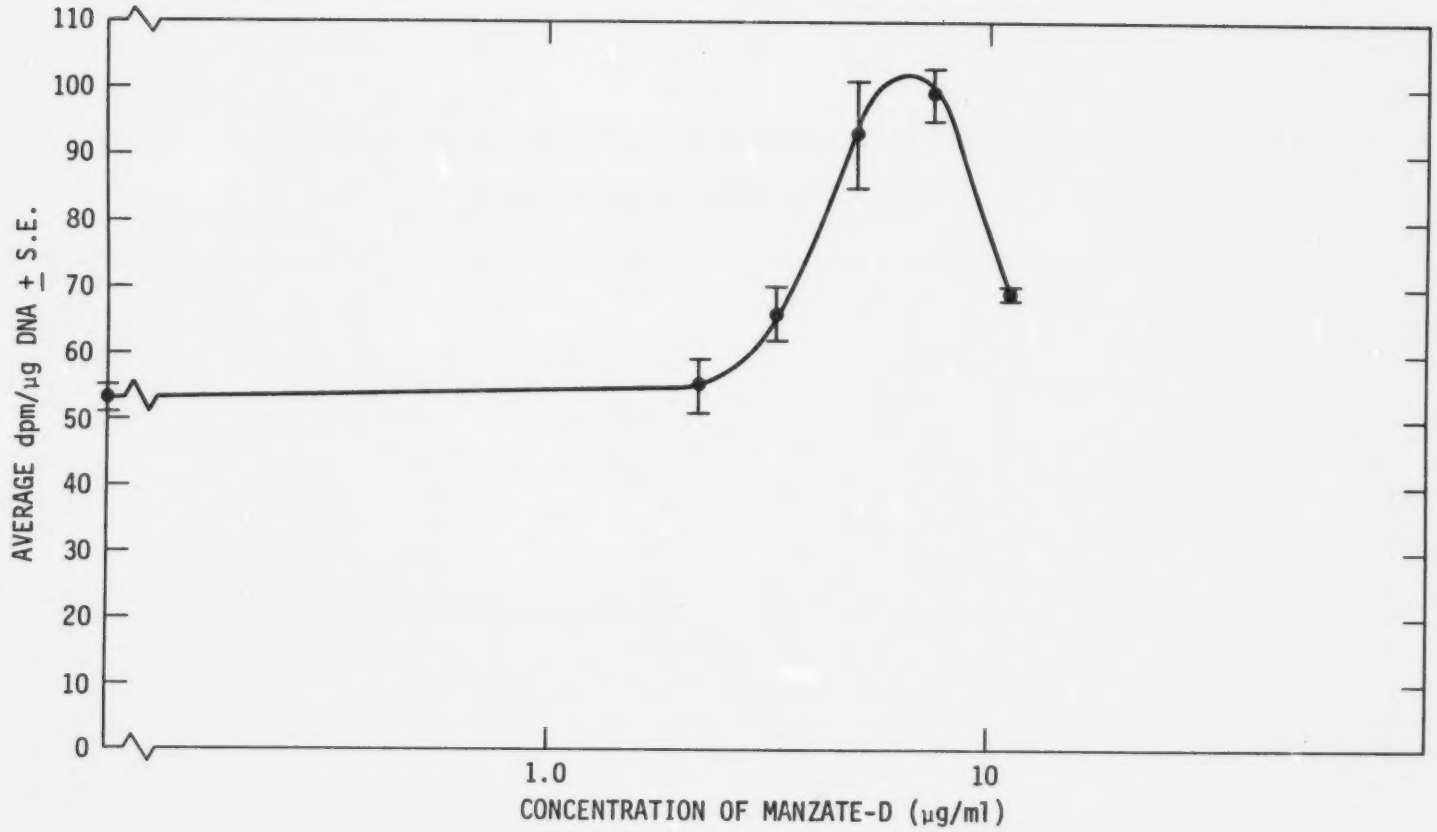

FIGURE 8 UNSCHEDULED DNA SYNTHESIS ASSAYS OF MANZATE-D WITHOUT METABOLIC ACTIVATION

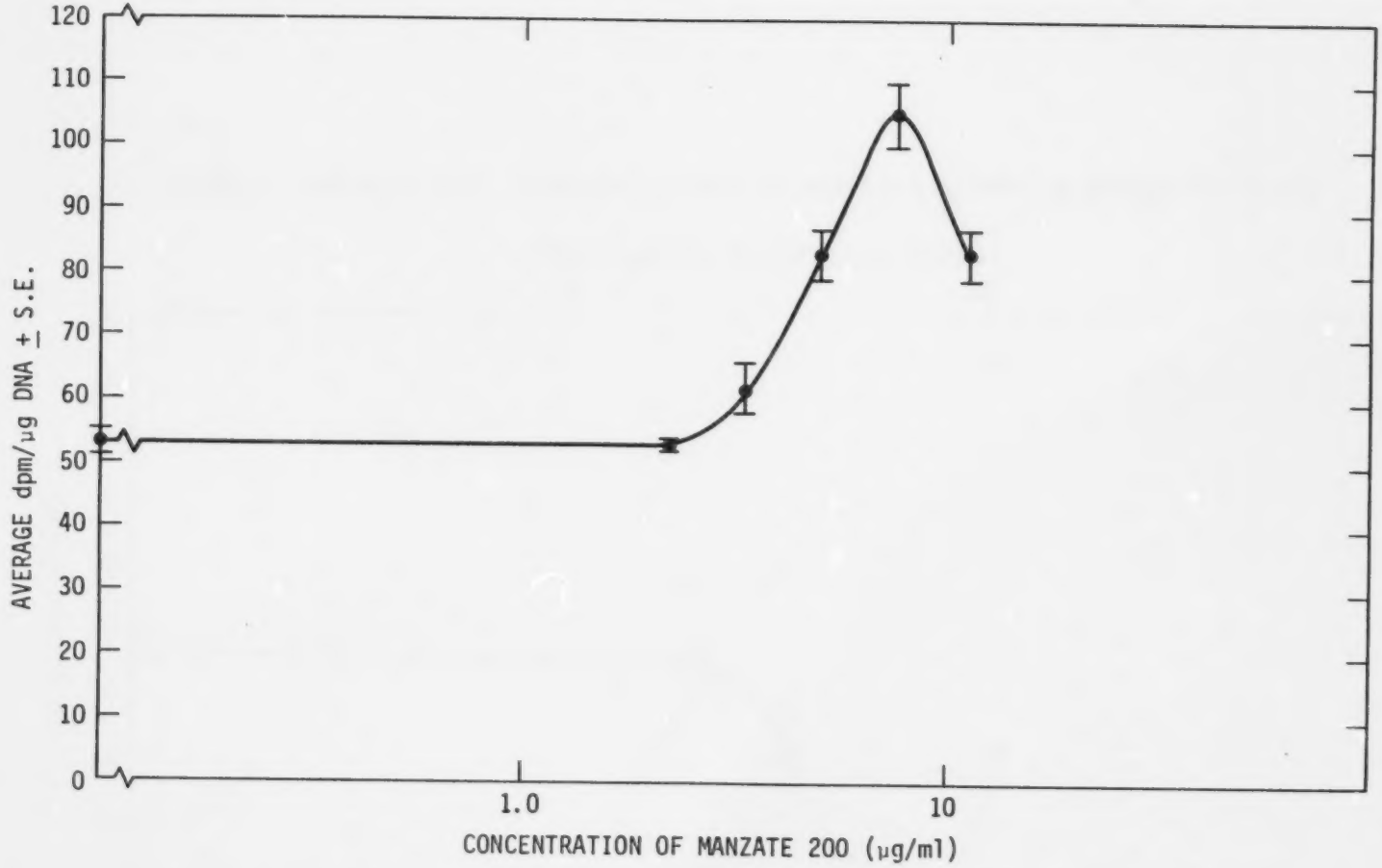

FIGURE 9 UNSCHEDULED DNA SYNTHESIS ASSAYS OF MANZATE 200 WITHOUT METABOLIC ACTIVATION

(manzate-D, $H = 26.57 \simeq \chi^2_5 > 15.09$; manzate 200, $F_{5,30} = 33.02 > 3.70$). The testing of manzate-D and manzate 200 with metabolic activation (Tables 101, 102, 106, and 107) failed to indicate increases in ^{3}H-TdR incorporation. Thus, UDS was induced by these compounds only in the absence of metabolic activation.

The initial results of the testing of zineb (Tables 129 and 130) suggested that this compound did not induce UDS in the absence of metabolic activation; however, a statistically significant elevation in ^{3}H-TdR uptake ($F_{4,25} = 10.90 > 4.18$) was observed at a concentration of 100 µg/ml with metabolic activation (Table 132). Because this was the only significant increase, a second assay of zineb with metabolic activation was conducted. The results of this test (Table 133) did not provide evidence for a dose-dependent increase in UDS. Although an apparent elevation in ^{3}H-TdR incorporation was observed, it was not statistically significant. Thus zineb did not appear to induce UDS.

The results of the testing of the following compounds did not indicate significant increases in UDS in either the presence or the absence of metabolic activation: biphenyl (Tables 74 to 77), chlordimeform (Tables 78 to 81), DL-cis/trans chrysanthemic acid (Tables 82 to 85), dithane M-22 (Tables 86 to 89), dithane M-45 (Tables 90 to 93), ethylchrysanthemate (Tables 94 to 97), NRDC-149 (Tables 108 to 111), permethrin (Tables 112 to 115), polyram (Tables 116 to 119), resemethrin (Tables 120 to 123), and sumithrin (Tables 124 to 127).

CONCLUSIONS

Our results indicate that none of the 15 pesticides tested in Phase III were mutagenic or genotoxic in all six of the *in vitro* assays. Only two pesticides were positive in more than one assay. They were manzate-D and manzate 200, which increased mitotic recombination in *S. cerevisiae* D3 and induced UDS in WI-38 cells. Dithane M-22, dithane M-45, ethylchrysanthemate, and zineb increased mitotic recombination. The *Salmonella*/microsome assay detected mutagenic activity in only one pesticide, bioallethrin. No pesticide was found to be mutagenic with *E. coli* WP2. DL-*cis*/*trans* chrysanthemic acid was genotixic in the relative toxicity assay.

It is recommended that these eight pesticides that were positive in at least one assay be considered for further study to more completely characterize their potential hazards to human health. Although a mutagenic response does not mean that a chemical is harmful to humans, the combination of six separate assay systems greatly enhances the probability of detecting potentially hazardous chemicals. It is apparent that no one assay is uniquely capable of detecting the spectrum of mutagenic events that different chemical structures may cause.

Table 1

FIFTEEN PESTICIDES EVALUATED BY SRI INTERNATIONAL FOR MUTAGENICITY

Common or Trade Name	Manufacturer	Purity	Batch or Lot No.	Supplier
Bioallethrin	S. B. Penick Co.	Tech. 93%	7806-RS	EPA
Biphenyl	Univ. of California at Riverside			EPA
Chlordimeform	Ciba-Geigy			EPA
DL-cis/trans Chrysanthemic acid	S. B. Penick Co.	93% (36.9% cis/ 63% trans)	8451-RW	EPA
Dithane M-22	Rohm and Haas			EPA
Dithane M-45	Rohm and Haas			EPA
Ethylchrysanthemate	Stauffer Chemical Co.		Lot 5272	EPA
Manzate-D	E. I. du Pont de Nemours & Co.			EPA
Manzate 200	du Pont			EPA
NRDC-149	ICI-America	90.5% (37.2% cis/ 53.6% trans)	BX-P14, PP383 2E	EPA
Permethrin	ICI-America	Tech. 90.4% (38.3% cis/52.3% trans)	BX-61223	EPA
Polyram	FMC Corporation	Tech.	C7606-29-B	EPA
Resmethrin	S. B. Penick Co.	90%	3521-LTV-3	EPA
Sumithrin		Tech. 94.4%		EPA
Zineb	FMC Corporation	Tech.	337	EPA

Table 2

IN VITRO MUTAGENESIS: SUMMARY DATA FOR EPA PESTICIDES

Pesticide	*Salmonella typhimurium* (His^+ Reversion)		*Escherichia coli* WP2 (Try^+ Reversion)		*Saccharomyces cerevisiae* D3 (Mitotic Recombinants)		*Escherichia coli* (Relative Toxicity)	*Bacillus subtilis* (Relative Toxicity)	UDS (DNA Repair)	
	-MA	+MA	-MA	+MA	-MA	+MA			-MA	+MA
Bioallethrin	-*	+	-	-	-	-	-	-	-	-
Biphenyl	-	-	-	-	-	-	-	-	-	-
Chlordimeform	-	-	-	-	-	-	-	-	-	-
DL-*cis*/*trans* Chrysanthemic acid	-	-	-	-	-	-	-	+	-	-
Dithane M-22	-	-	-	-	+	-	-	-	-	-
Dithane M-45	-	-	-	-	+	+	-	-	-	-
Ethylchrysanthemate	-	-	-	-	+	+	-	-	-	-
Manzate-D	-	-	-	-	+	+	-	-	+	-
Manzate 200	-	-	-	-	+	+	-	-	+	-
NRDC-149	-	-	-	-	-	-	-	-	-	-
Permethrin	-	-	-	-	-	-	-	-	-	-
Polyram	-	-	-	-	-	-	-	-	-	-
Resmethrin	-	-	-	-	-	-	-	-	-	-
Sumithrin	-	-	-	-	-	-	-	-	-	-
Zineb	-	-	-	-	+	+	-	-	-	-

*Negative response, -; positive response, +.

Table 3

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI
BIOALLETHRIN

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		9	5	19	123	29
	+		7	4	42	150	38
Positive controls							
2-Anthramine	+	2.5	149		422	890	
	+	10					245
9-Aminoacridine	-	50		70			
Bioallethrin	-	50	15	6	13	86	20
	-	100	7	4	12	97	22
	-	500	10	4	19	88	21
	-	1000	7	4	10	97	17
	-	2500	7	3	9	99	13
	-	5000	6	4	8	104	22
	-	10000	11	4	15	98	21
	+	50	7	7	41	135	19
	+	100	12	6	38	165	28
	+	500	12	8	36	225	23
	+	1000	5	11	33	296	37
	+	2500	10	6	25	282	20
	+	5000	16	6	29	276	32
	+	10000	9	8	29	294	30

Table 4

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

BIOALLETHRIN

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		16	7	21	106	29
	+		13	10	27	96	59
Positive controls							
2-Anthramine	-	1.0			23	153	
	+	1.0			154	437	
	-	2.5	33	15			
	+	2.5	98	100			
	-	10					87
	+	10					311
9-Aminoacridine	-	50		231			
Sodium azide	-	0.5	139			329	
2-Nitrofluorene	-	5.0			502		
AF2	-	0.1					736
Bioallethrin	-	500	18	2	22	81	40
	-	1000	14	9	22	78	40
	-	2500	25	5	21	101	31
	-	5000	15	5	24	103	36
	-	10000	20	3	28	123	39
	-	20000	20	1	26	144	48
	+	500	9	4	39	166	58
	+	1000	9	6	32	221	48
	+	2500	10	5	32	206	40
	+	5000	10	9	38	201	61
	+	10000	14	7	34	206	56
	+	20000	14	2	34	205	40

Table 5

IN VITRO ASSAYS OF SALMONELLA TYPHIMURIUM STRAIN TA100
BIOALLETHRIN

Experiment 3

Compound	Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate			
			Plate 1	Plate 2	Plate 3	Average
Negative control (DMSO)	-		149	115	105	123
	+		92	110	92	98
Positive control						
Sodium azide	-	0.5	373	352	NT*	363
2-Anthramine	-	1.0	103	87	NT	95
	+	1.0	224	290	NT	257
Bioallethrin	+	10	93	C†	90	92
	+	25	100	109	131	113
	+	50	126	125	115	122
	+	75	111	124	113	116
	+	100	143	114	122	126
	+	250	136	172	136	148
	+	500	166	201	185	184
	+	750	170	174	190	178
	+	1000	167	182	182	177
	+	2500	172	215	251	213

*NT, not tested.

†C, contaminated.

Table 6

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

BIPHENYL

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		21	3	24	121	31
	+		5	7	27	119	36
Positive controls							
2-Anthramine	+	2.5	146		554	862	
	+	10					
9-Aminoacridine	-	100		609			117
Biphenyl	-	1	18	4	29	111	28
	-	10	24	2	19	101	33
	-	50	11	3	14	107	18
	-	100	11	2	14	80	24
	-	500	17	5	8	88	24
	-	1000	12	3	10	78	29
	+	1	17	8	33	91	26
	+	10	12	6	24	102	22
	+	50	15	6	36	74	35
	+	100	7	4	34	84	40
	+	500	9	6	22	67	38
	+	1000	2	4	18	70	26

Table 7

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

BIPHENYL

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella cyphimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		19	4	19	121	29
	+		10	5	36	123	34
Positive controls							
2-Anthramine	+	2.5	151		743	1961	
	+	10					
9-Aminoacridine	-	50		128			364
Biphenyl	-	100	16	6	15	104	33
	-	250	13	7	16	97	29
	-	500	12	1	18	82	24
	-	1000	7	3	15	29T*	32
	-	2500	T	T	T	T	16
	-	5000	T	T	T	T	T
	+	100	4	5	31	98	32
	+	250	5	12	26	105	36
	+	500	5	2	29	63	38
	+	1000	3	8	20	T	26
	+	2500	T	T	14	T	22
	+	5000	T	T	T	T	T

* T, toxic.

Table 8

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

CHLORDIMEFORM

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		21	3	24	121	31
	+		5	7	27	119	36
Positive controls							
2-Anthramine	+	2.5	146		554	862	
	+	10					
9-Aminoacridine	-	100		609			117
Chlordimeform	-	1	15	6	26	115	30
	-	10	13	3	30	94	24
	-	50	15	3	22	110	26
	-	100	25	6	25	95	23
	-	500	24	5	21	117	25
	-	1000	11	4	17	90	27
	+	1	7	7	40	83	20
	+	10	11	8	35	101	32
	+	50	9	6	28	82	29
	+	100	8	6	36	82	26
	+	500	7	4	22	72	25
	+	1000	9	7	35	97	31

Table 9

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

CHLORDIMEFORM

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		20	14	19	119	32
	+		16	11	25	125	48
Positive controls							
2-Anthramine	+	2.5	142		995	1598	
	+	10					212
9-Aminoacridine	-	50		214			
Chlordimeform	-	100	20	12	20	125	48
	-	500	24	13	15	81	54
	-	1000	19	6	12	116	29
	-	2500	21	8	9	108	21
	-	5000	T*	T	T	T	T
	-	7500	T	T	T	T	T
	+	100	10	7	18	137	52
	+	500	6	16	17	102	38
	+	1000	8	3	12	110	33
	+	2500	5	8	15	118	39
	+	5000	7	12	13	83	22
	+	7500	T	T	10	81	T

* T, toxic.

Table 10

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

DL-cis/trans CHRYSANTHEMIC ACID

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		5	4	18	92	25
	+		4	3	22	109	48
Positive controls							
2-Anthramine	+	2.5	303		1381	1552	803
9-Aminoacridine	-	100		1508			
DL-cis/trans chrysanthemic acid	-	1	8	3	15	108	28
	-	10	9	4	15	101	31
	-	50	8	6	19	78	41
	-	100	11	2	20	74	29
	-	500	4	3	11	87	39
	-	1,000	8	4	4	78	33
	+	1	10	5	26	83	40
	+	10	9	8	31	86	49
	+	50	13	4	29	85	52
	+	100	8	6	30	88	44
	+	500	14	5	31	104	30
	+	1,000	8	10	35	110	39

Table 11

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

DL-cis/trans CHRYSANTHEMIC ACID

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		21	6	28	123	32
	+		10	4	23	138	24
Positive controls							
2-Anthramine	+	2.5	280		1073	1650	
	+	10					172
9-Aminoacridine	-	100		1018			
DL-cis/trans chrysanthemic acid	-	100	14	5	23	135	25
	-	500	14	5	40	127	17
	-	1,000	12	5	14	100	23
	-	2,500	2	T*	12	39	19
	-	5,000	T	T	T	T	23
	-	10,000	T	T	T	T	T
	+	100	9	4	36	127	22
	+	500	12	7	28	151	30
	+	1,000	16	3	25	134	27
	+	2,500	13	6	26	110	27
	+	5,000	5	3	18	142	23
	+	10,000	7	4	21	112	18

* T, toxic.

Table 12

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

DITHANE M-22

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		21	5	46	125	25
	+		10	8	55	101	48
Positive controls							
2-Anthramine	+	2.5	270				
	+	10			1239	1508	803
9-Aminoacridine	-	100		939			
Dithane M-22	-	1	31	4	36	101	26
	-	10	18	3	34	85	30
	-	50	5	T*	3	T	25
	-	100	T	T	T	T	T
	-	500	T	T	T	T	T
	-	1000	T	T	T	T	T
	+	1	16	4	54	84	50
	+	10	8	7	34	95	46
	+	50	8	4	48	101	40
	+	100	8	3	46	77	25
	+	500	T	T	T	T	T
	+	1000	T	T	T	T	T

* T, toxic.

Table 13

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI
DITHANE M-22

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Average Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		26	8	13	121	55
	+		12	8	32	123	70
Positive controls							
Sodium azide	-	0.5	182			277	
	-	1.0	244*			375*	
9-Aminoacridine	-	50		127			
2-Nitrofluorene	-	5			513		
AF2	-	0.1					913
2-Anthramine	-	1.0			27*	145*	
	+	1.0			755*	1392*	
	-	2.5	17	9			
	+	2.5	196	148			
	-	10.0					65
	+	10.0					593
Dithane M-22	-	0.5*	12	8	17	96	47
	-	1.0	18	6	10	91	54
	-	5.0	19	6	13	107	50
	-	10.0	16	6	15	100	47
	-	25.0	12	6	11	103	40
	-	50.0	T†	T	T	T	T
	+	50.0	13	5	25	117	68
	+	100.0	9	6	23	96	68
	+	150.0	13	4	25	113	67
	+	200.0	10	5	16	111	78
	+	250.0	8	7	14	100	66
	+	300.0	10	5	15	83	87
	+	350.0	7	8	16	T	79

* Results from one experiment.
† T, toxic.

Table 14

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

DITHANE M-45

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		21	5	46	125	25
	+		10	8	55	101	48
Positive controls							
2-Anthramine	+	2.5	270		1239	1508	
	+	10					803
9-Aminoacridine	-	100		939			
Dithane M-45	-	1	20	3	50	134	25
	-	10	26	3	28	101	19
	-	50	T*	2	4	T	23
	-	100	T	T	T	T	18
	-	500	T	T	T	T	T
	+	1	10	8	43	97	28
	+	10	8	5	46	100	31
	+	50	2	4	36	106	29
	+	100	T	4	52	88	36
	+	500	T	T	T	T	T

* T, toxic.

Table 15

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

DITHANE M-45

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		22	3	17	130	54
	+		15	3	19	136	45
Positive controls							
2-Anthramine	+	2.5	179		832	2621	
	+	10					294
Sodium azide	-	0.5	212			435	
9-Aminoacridine	-	50		123			
Dithane M-45	-	0.5	25	8	14	137	35
	-	1.0	20	7	18	117	37
	-	5.0	21	9	17	103	30
	-	10.0	25	4	11	108	32
	-	25.0	12	3	9	66T*	34
	-	50.0	T	7	10	T	33
	+	0.5	15	7	22	112	46
	+	1.0	19	6	26	111	40
	+	5.0	9	2	28	112	46
	+	10.0	16	5	20	118	45
	+	25.0	7	9	25	120	42
	+	50.0	8	6	19	137	41

* T, toxic.

Table 16

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

DITHANE M-45

Experiment 3

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Average Histidine Revertants per Plate TA1535	TA1537	TA98	TA100	E. coli Tryptophan Revertants per Plate WP2
Negative control	-		19	5	14	136	57
	+		11	5	25	137	45
Positive controls							
2-Anthramine	-	2.5	13†	4†	21†	132†	
	+	2.5	252	225†	1179	1669	
	-	10					42†
	+	10					874†
Sodium azide	-	0.5	183†			293†	
9-Aminoacridine	-	50		159			
2-Nitrofluorene	-	5.0			547		
AF2	-	0.1					1184†
Dithane M-45	-	0.5	19	5	14	135	40
	-	1.0	18	4	16	122	40
	-	5.0	19	7	16	112	40
	-	10.0	22	7	13	121	47
	-	25.0	10	3	10	85	41
	-	50.0	T*	7	8	T	43
	-	75.0†	T	2	T	T	49
	+	25.0†	16	2	33	135	51
	+	50.0	12	5	24	103	37
	+	75.0†	10	4	22	108	56
	+	100.0	9	8	22	104	39
	+	150.0	10	6	13	92	39
	+	200.0	10	9	16	92	36
	+	250.0	T	T	T	T	T

* T, toxic.

† Data from [illegible]

Table 17

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

ETHYLCHRYSANTHEMATE

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		20	7	24	90	29
	+		15	6	32	114	35
Positive controls							
2-Anthramine	+	2.5	156		444	590	112
9-Aminoacridine	-	100		1725			
Ethylchrysanthemate	-	1	13	9	18	83	41
	-	10	26	5	28	87	34
	-	50	14	7	29	100	21
	-	100	12	6	25	107	24
	-	500	16	5	17	77	23
	-	1000	14	4	21	110	23
	+	1	7	8	39	70	29
	+	10	9	6	31	75	39
	+	50	10	7	27	98	26
	+	100	10	5	37	63	36
	+	500	11	8	23	78	19
	+	1000	7	6	29	81	26

Table 18

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

ETHYLCHRYSANTHEMATE

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		21	6	28	123	32
	+		10	4	23	138	24
Positive controls							
2-Anthramine	+	2.5	280		1073	1650	
	+	10					172
9-Aminoacridine	-	100		1018			
Ethylchrysanthemate	-	100	11	3	17	96	30
	-	500	14	3	14	99	21
	-	1000	12	2	7	98	16
	-	2500	8	3	16	104	15
	-	5000	5	T*	7	126	18
	-	10000	T	T	6	79	15
	+	100	6	6	23	94	28
	+	500	10	5	22	88	25
	+	1000	6	7	14	102	30
	+	2500	7	4	19	109	31
	+	5000	8	3	22	82	30
	+	10000	5	3	19	86	33

* T, toxic.

Table 19

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

MANZATE-D

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Average Histidine Revertants per Plate TA1535	TA1537	TA98	TA100	E. coli Tryptophan Revertants per Plate WP2
Negative control	-		20	6	22	110	30
	+		12	8	24	114	46
Positive controls							
2-Anthramine	-	1.0			33	110	
	+	1.0			230	1064	
	-	2.5	18	8			
	+	2.5	181	144			
	-	10					73
	+	10					153
9-Aminoacridine	-	50		139			
Sodium azide	-	0.5	213			289	
2-Nitrofluorene	-	5.0			407		
AF2	-	0.1					755
Manzate-D	-	0.5	16	8	22	115	32
	-	1.0	17	3	24	110	26
	-	5.0	22	7	18	87	23
	-	10.0	10	6	18	83	24
	-	50.0	T*	T	T	T	26
	-	100.0	T	T	T	T	T
	+	1.0†	18	6	28	132	40
	+	5.0†	5	8	38	89	34
	+	10.0	16	7	24	92	36
	+	50.0	14	5	25	110	35
	+	100.0	12	5	28	82	36
	+	500.0	T	T	T	T	T

* T, toxic.

† Data from one experiment.

Table 20

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

MANZATE-D

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		14	8	38	102	27
	+		8	8	30	107	46
Positive controls							
2-Anthramine	-	1.0			26	101	
	+	1.0			315	515	
	-	2.5	19	8			
	+	2.5	123	11			
	-	10					24
	+	10					207
9-Aminoacridine	-	50		200			
Sodium azide	-	1.0	246			317	
2-Nitrofluorene	-	5.0			441		
AF2	-	0.1					773
Manzate-D	-	0.5	11	7	27	80	17
	-	1.0	15	6	29	78	20
	-	5.0	15	7	27	89	21
	-	10.0	12	5	28	75	27
	-	25.0	11	8	14	68	13
	-	50.0	T*	T	T	T	14
	-	75.0	T	T	T	T	T
	+	50.0	9	10	29	104	30
	+	100.0	12	7	25	73	30
	+	200.0	8	9	25	78	32
	+	300.0	3	3	19	T	T
	+	400.0	T	T	T	T	T
	+	500.0	T	T	T	T	T

* T, toxic.

Table 21

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

MANZATE 200

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Average Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		20	10	28	113	52
	+		11	8	24	114	46
Positive controls							
2-Anthramine	-	1.0			27	123	
	+	1.0			535	918	
	-	2.5	20	11			
	+	2.5	133	91			
	-	10					56
	+	10					259
9-Aminoacridine	-	50		175			
Sodium azide	-	1.0	245			346	
2-Nitrofluorene	-	5.0			530		
AF2	-	0.1					796
Manzate 200	-	0.5*	18	7	24	97	23
	-	1.0	19	10	26	108	43
	-	5.0	15	7	19	102	50
	-	10.0	16	4	22	100	32
	-	50.0	T†	T	T	T	37
	-	100.0	T	T	T	T	T
	+	1.0	11	7	28	99	35
	+	5.0*	9	10	31	109	40
	+	10.0	10	6	33	102	30
	+	50.0	7	5	34	95	35
	+	100.0	8	4	21	83	36
	+	500.0	T	T	T	T	T

* Data from one experiment.
† T, toxic.

Table 22

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

MANZATE 200

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		14	8	38	102	27
	+		8	8	30	107	46
Positive controls							
2-Anthramine	-	1.0			26	101	
	+	1.0			315	515	
	-	2.5	19	8			
	+	2.5	123	111			
	-	10					24
	+	10					207
9-Aminoacridine	-	50		200			
Sodium azide	-	1.0	246			317	
2-Nitrofluorene	-	5.0			441		
AF2	-	0.1					773
Manzate 200	-	0.5	21	9	33	100	20
	-	1.0	16	9	28	100	33
	-	5.0	16	9	25	90	29
	-	10.0	19	6	25	98	31
	-	25.0	6	9	27	85	25
	-	50.0	T*	T	T	T	29
	-	75.0					29
	+	75.0	8	9	46	72	31
	+	100.0	13	9	32	76	29
	+	150.0	10	9	29	74	29
	+	200.0	4	5	17	63	29
	+	300.0	4	7	23	77	26
	+	500.0	T	T	T	T	T

Table 23

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

NRDC-149

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		20	11	18	118	45
	+		12	7	20	104	43
Positive controls							
2-Anthramine	+	2.5	198		352	398	
	+	10					105
9-Aminoacridine	-	100		472			
NRDC-149	-	1	25	8	25	130	38
	-	10	28	10	23	123	30
	-	50	24	8	17	115	39
	-	100	33	8	16	115	41
	-	500	24	6	15	129	31
	-	1000	48	7	26	131	39
	+	1	11	4	42	117	45
	+	10	12	7	28	110	37
	+	50	8	7	32	139	41
	+	100	12	11	34	116	43
	+	500	11	10	32	124	36
	+	1000	10	5	40	113	42

Table 24

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

NRDC-149

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		20	6	20	123	50
	+		19	12	21	135	61
Positive controls							
2-Anthramine	-	1.0			26	125	
	+	1.0			232	527	
	-	2.5	23	8			
	+	2.5	182	154			
	-	10					45
	+	10					231
Sodium azide	-	0.5	172			286	
9-Aminoacridine	-	50		339			
AF2	-	0.1					820
2-Nitrofluorene	-	5.0			394		
NRDC-149	-	500	18	8	22	108	57
	-	1000	29	8	6	99	49
	-	2500	19	3	26	112	41
	-	5000	17	5	20	100	32
	-	7500	14	5	15	104	37
	-	10000	17	5	18	110	29
	+	500	14	4	19	94	43
	+	1000	14	7	20	101	45
	+	2500	14	7	27	106	45
	+	5000	12	6	18	96	40
	+	7500	11	3	21	100	32
	+	10000	7	6	17	85	22

Table 25

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

NRDC-149

Experiment 3

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		22	3	17	130	54
	+		15	3	19	136	45
Positive controls							
2-Anthramine	-	2.5	28	7	20	125	
	+	2.5	179	177	832	2621	
	-	10					43
	+	10					294
9-Aminoacridine	-	50		123			
Sodium Azide	-	0.5	212			435	
NRDC-149	-	5000	22	9	17	125	37
	-	6000	28	10	16	119	35
	-	7000	26	11	17	121	34
	-	8000	20	11	23	116	32
	-	9000	24	9	18	120	32
	-	10000	20	5	11	124	29
	+	5000	21	9	28	133	39
	+	6000	22	7	25	130	42
	+	7000	20	8	24	136	45
	+	8000	25	10	17	129	37
	+	9000	26	8	23	135	45
	+	10000	22	3	26	130	39

Table 26

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

PERMETHRIN

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate TA1535	TA1537	TA98	TA100	E. coli Tryptophan Revertants per Plate WP2
Negative control	-		14	8	14	94	19
	+		10	3	19	98	22
Positive control							
2-Anthramine	+	2.5	55		178	320	
	+	10					104
9-Aminacridine	-	100		645			
Permethrin	-	1	15	1	14	66	24
	-	10	15	5	19	98	17
	-	50	22	3	10	109	25
	-	100	18	4	8	98	18
	-	500	16	3	19	115	30
	-	1000	9	5	11	93	15
	+	1	14	5	21	90	25
	+	10	11	10	27	100	16
	+	50	10	7	16	72	20
	+	100	9	6	18	83	27
	+	500	8	5	17	91	24
	+	1000	8	9	22	116	28

Table 27

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

PERMETHRIN

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Average Histidine Revertants per Plate TA1535	TA1537	TA98	TA100	E. coli Tryptophan Revertants per Plate WP2
Negative control	-		15	6	15	100	37
	+		14	9	20	108	48
Positive controls							
2-Anthramine	-	1.0			26 *	125 *	
	+	1.0			232 *	527 *	
	-	2.5	23 *	8 *			
	+	2.5	182 *	154 *			
	-	10					45 *
	+	10					231 *
Sodium azide	-	0.5	172 *				
9-Aminoacridine	-	50		339 *			
2-Nitrofluorene	-	5.0			394 *		
AF2	-	0.1					820 *
Permethrin	-	100	13	8	11	98	44
	-	500	12	7	12	93	45
	-	1000	13	5	15	73	41
	-	2500	16	8	14	78	37
	-	5000	15	6	10	86	41
	-	7500	17	6	16	80	48
	+	100	8	7	21	86	45
	+	500	11	6	16	102	54
	+	1000	7	8	14	99	57
	+	2500	8	12	18	112	42
	+	5000	9	9	16	103	45
	+	7500	12	12	18	98	48

* Results from one experiment.

Table 28

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

POLYRAM

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		14	8	14	94	19
	+		10	3	19	98	22
Positive controls							
2-Anthramine	+	2.5	55		178	320	
	+	10					
9-Aminoacridine	-	100		645			104
Polyram	-	1	8	2	23	81	25
	-	10	8	2	15	72	13
	-	50	T*	3	10	T	20
	-	100	T	T	T	T	T
	-	500	T	T	T	T	T
	-	1000	T	T	T	T	T
	+	1	9	3	19	85	33
	+	10	5	7	21	75	19
	+	50	7	7	22	82	21
	+	100	1	5	16	80	26
	+	500	2	3	24	76	26
	+	1000	T	T	T	T	T

* T, toxic.

Table 29

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

POLYRAM

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptopha Revertant per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		22	15	31	130	56
	+		10	5	36	123	34
Positive controls							
2-Anthramine	+	2.5	151		743	1961	
	+	10					364
9-Aminoacridine	-	50		165			
Polyram	-	0.5	15	16	35	134	62
	-	1.0	27	17	22	144	42
	-	5.0	15	13	32	118	56
	-	10.0	15	7	29	96	39
	-	25.0	19	13	24	111	59
	-	50.0	T*	9	30	T	57
	+	25.0	16	7	27	138	38
	+	50.0	13	7	33	105	41
	+	100.0	10	6	23	128	51
	+	250.0	15	6	26	130	43
	+	500.0	9	4	20	125	35
	+	1000.0	0	0	0	0	T

* T, toxic.

Table 30

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

RESMETHRIN

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		20	7	24	90	29
	+		15	6	32	114	35
Positive controls							
2-Anthramine	+	2.5	156		444	590	112
9-Aminoacridine	-	100		1725			
Resmethrin	-	1	14	7	15	96	23
	-	10	17	5	19	79	24
	-	50	18	6	5	80	23
	-	100	18	5	22	96	22
	-	500	12	2	18	103	19
	-	1000	20	9	27	77	29
	+	1	9	6	31	82	40
	+	10	10	4	24	80	33
	+	50	8	9	38	94	25
	+	100	9	5	27	87	45
	+	500	9	6	35	87	22
	+	1000	7	5	48	82	32

Table 31

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

RESMETHRIN

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		21	6	28	123	32
	+		10	4	23	138	24
Positive controls							
2-Anthramine	+	2.5	280		1073	1650	
	+	10					172
9-Aminoacridine	-	100		1018			
Resmethrin	-	100	9	2	26	126	28
	-	500	11	7	30	146	32
	-	1000	13	4	20	137	32
	-	2500	8	5	16	113	28
	-	5000	6	2	28	158	29
	-	10000	5	1	26	138	25
	+	100	6	8	33	164	29
	+	500	5	3	42	163	29
	+	1000	6	5	39	169	35
	+	2500	9	11	37	167	37
	+	5000	10	7	38	155	32
	+	10000	12	4	42	128	26

Table 32

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

SUMITHRIN

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		21	3	24	121	31
	+		5	7	27	119	36
Positive controls							
2-Anthramine	+	2.5	146		554	862	
	+	10					117
9-Aminoacridine	-	100		609			
Sumithrin	-	1	13	2	29	84	32
	-	10	18	8	36	67	40
	-	50	13	2	24	91	36
	-	100	13	1	28	96	25
	-	500	15	5	18	127	29
	-	1000	14	7	18	115	28
	+	1	16	4	25	89	45
	+	10	14	7	39	85	32
	+	50	17	5	31	93	44
	+	100	7	4	36	113	42
	+	500	8	2	28	108	40
	+	1000	14	6	33	138	38

Table 33

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

SUMITHRIN

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		7	6	9	77	23
	+		9	6	19	80	34
Positive controls							
2-Anthramine	+	2.5	149		663	995	
	+	7.5					179
9-Aminoacridine	-	50		190			
Sumithrin	-	100	12	8	6	60	22
	-	500	8	8	5	76	27
	-	1000	12	7	4	84	35
	-	2500	11	7	10	61	37
	-	5000	15	4	10	71	29
	-	7500	18	3	5	75	30
	+	100	5	6	16	59	29
	+	500	9	5	20	79	26
	+	1000	9	6	14	70	27
	+	2500	6	4	19	78	24
	+	5000	12	9	15	85	25
	+	7500	20	7	37	121	35

Table 34

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

SUMITHRIN

Experiment 3

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate TA1535	TA1537	TA98	TA100	E. coli Tryptophan Revertants per Plate WP2
Negative control	-		22	5	11	130	44
	+		15	14	23	136	57
Positive controls							
2-Anthramine	+	2.5	179		1078	2621	
	+	10					126
9-Aminoacridine	-	50		140			
Sodium azide	-	0.5	212			435	
Sumithrin	-	5000	12	8	12	140	45
	-	6000	19	5	17	136	43
	-	7000	16	3	12	144	38
	-	8000	24	8	14	164	51
	-	9000	23	7	15	135	55
	-	10000	27	6	12	159	54
	+	5000	18	7	28	140	43
	+	6000	11	8	35	130	44
	+	7000	17	15	32	128	63
	+	8000	9	4	22	155	62
	+	9000	12	5	35	95	56
	+	10000	21	4	30	125	52

Table 35

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

ZINEB

Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate TA1535	TA1537	TA98	TA100	E. coli Tryptophan Revertants per Plate WP2
Negative control	-		14	8	14	94	19
	+		10	3	19	98	22
Positive controls							
2-Anthramine	+	2.5	55		178	320	
	+	10					104
9-Aminoacridine	-	100		645			
Zineb	-	1	9	5	12	74	14
	-	10	11	4	10	100	13
	-	50	7	4	16	83	19
	-	100	T*	T	3	T	18
	-	500	T	T	T	T	T
	-	1000	T	T	T	T	T
	+	1	5	8	23	98	20
	+	10	10	8	24	87	11
	+	50	9	3	22	103	26
	+	100	10	8	24	95	24
	+	500	2	4	15	72	28
	+	1000	3	6	15	55	T

* T, toxic.

Table 36

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

ZINEB

Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		20	14	19	119	32
	+		8	8	30	107	46
Positive controls							
2-Anthramine	-	1.0			26	101	
	+	1.0			315	515	
	-	2.5	19	8			
	+	2.5	123	111			
	-	10					24
	+	10					207
9-Aminoacridine	-	50		200			
Sodium azide	-	1.0	246			317	
2-Nitrofluorene	-	5.0			441		
AF2	-	0.1					773
Zineb	-	0.5	19	13	16	118	55
	-	1.0	13	15	16	135	40
	-	10.0	23	16	20	116	49
	-	25.0	20	18	21	110	51
	-	50.0	17	17	17	102	34
	-	75.0	18	11	12	113	39
	+	5.0	8	5	30	101	38
	+	10.0	12	9	37	137	44
	+	25.0	16	6	30	126	40
	+	50.0	16	10	36	117	42
	+	100.0	6	6	40	91	31
	+	500.0	11	8	22	91	24

Table 37

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

ZINEB

Experiment 3

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Salmonella typhimurium Histidine Revertants per Plate				E. coli Tryptophan Revertants per Plate
			TA1535	TA1537	TA98	TA100	WP2
Negative control	-		9	5	19	95	20
	+		8	10	31	127	48
Positive controls							
2-Anthramine	-	2.5	13	4	21	132	
	+	2.5	252	225	1179	1669	
	-	10					42
	+	10					874
9-Aminoacridine	-	50		159			
Sodium azide	-	0.5	183			293	
2-Nitrofluorene	-	5.0			549		
AF2	-	0.1					1184
Zineb	-	0.5	8	3	13	105	13
	-	1.0	9	5	18	104	19
	-	10.0	12	1	10	107	25
	-	25.0	10	5	12	102	26
	-	50.0	8	3	16	T*	32
	-	75.0	9	T	19	T	25
	-	100.0	T	T	14	T	T
	+	50.0	13	7	30	110	29
	+	100.0	9	6	33	97	32
	+	250.0	10	8	31	90	41
	+	500.0	9	3	30	87	29
	+	750.0	3	7	15	T	T
	+	1000.0	T	T	15	T	T

* T, toxic.

Table 38

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
BIOALLETHRIN

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.0	100	2	4.0
	+		4.7	100	3	6.4
Positive control	-	0.04	5.8	116	630	1100
1,2,3,4-Diepoxybutane	+	0.04	4.1	87	435	1100
Bioallethrin	-	0.1	6.3	126	8	13
	-	0.5	6.8	136	5	7.4
	-	1.0	4.6	92	6	13
	-	5.0	6.8	136	4	5.9
	+	0.1	3.7	79	4	11
	+	0.5	6.1	130	3	4.9
	+	1.0	5.3	113	5	9.4
	+	5.0	6.8	145	7	10

Table 39

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
BIOALLETHRIN

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		7.0	100	6	8.6
	+		6.5	100	7	11
Positive control 1,2,3,4-Diepoxybutane	-	0.04	7.5	107	738	980
	+	0.04	7.1	109	778	1100
Bioallethrin	-	1.0	5.5	79	8	15
	-	2.0	6.6	94	3	4.6
	-	4.0	4.6	66	6	13
	-	5.0	6.3	90	3	4.8
	+	1.0	4.2	65	5	12
	+	2.0	6.1	94	4	6.6
	+	4.0	6.3	97	6	9.5
	+	5.0	9.4	145	11	12

Table 40

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

BIPHENYL

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.0	100	2	4.0
	+		4.7	100	3	6.4
Positive control 1,2,3,4-Diepoxybutane	-	0.04	5.8	116	630	1100
	+	0.04	4.1	87	435	1100
Biphenyl	-	0.1	7.3	146	2	2.7
	-	0.5	5.5	110	3	5.5
	-	1.0	6.8	136	1	1.5
	-	5.0	4.3	86	2	4.7
	+	0.1	5.2	111	5	9.6
	+	0.5	6.3	134	5	7.9
	+	1.0	5.6	119	1	1.8
	+	5.0	3.2	68	2	6.3

Table 41

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

BIPHENYL

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		7.0	100	6	8.6
	+		6.5	100	7	10.8
Positive control 1,2,3,4-Diepoxybutane	-	0.04	7.5	107	738	980
	+	0.04	7.1	109	778	1100
Biphenyl	-	1.0	3.8	54	8	21
	-	2.0	6.5	93	3	4.6
	-	4.0	3.9	56	2	5.1
	-	5.0	3.7	53	5	14
	+	1.0	3.2	49	3	9.4
	+	2.0	5.2	80	3	5.8
	+	4.0	6.4	98	4	6.3
	+	5.0	3.3	51	3	9.1

Table 42

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

CHLORDIMEFORM

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.9	100	5.0	8.5
	+		6.9	100	5.5	8.0
Positive control 1,2,3,4-Diepoxybutane	-	0.025	5.9	100	880.0	1500
	+	0.025	5.0	72	807.5	1600
Chlordimeform	-	0.08	6.2	105	5.0	8.1
	-	0.10	6.3	107	3.0	4.8
	-	0.12	7.3	124	8.0	11
	-	0.14	7.3	124	9.0	12
	+	0.8	8.3	120	6.0	7.2
	+	0.10	7.9	114	8.0	10
	+	0.12	8.5	123	1.0	1.2
	+	0.14	6.1	88	9.0	15

Table 43

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

CHLORDIMEFORM

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.6	100	6.5	12
	+		7.0	100	4.5	6.4
Positive control 1,2,3,4-Diepoxybutane	-	0.025	8.4	150	485.0	580
	+	0.025	7.7	110	650.0	840
Chlordimeform	-	0.08	6.0	107	10.0	17
	-	0.10	5.2	93	5.0	9.6
	-	0.12	3.0	54	5.0	17
	-	0.14	T*	T	T	T
	-	0.16	T	T	T	T
	+	0.08	7.6	109	11.0	15
	+	0.10	4.7	67	2.0	4.3
	+	0.12	4.2	60	8.0	19
	+	0.14	3.3	47	5.0	15
	+	0.16	2.5	36	2.0	80
	+	0.18	T	T	T	T

* T, toxic.

Table 44

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

DL-cis/trans CHRYSANTHEMIC ACID

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.6	100	6.5	12
	+		7.0	100	4.5	6.4
Positive control 1,2,3,4-Diepoxybutane	-	0.025	8.4	150	485.0	580
	+	0.025	7.7	110	650.0	840
DL-cis/trans chrysanthemic acid	-	0.5	7.6	136	11.0	15
	-	0.6	7.9	141	6.0	7.6
	-	0.7	5.9	105	13.0	22
	-	0.8	T*	T	T	T
	-	0.9	T	T	T	T
	+	0.5	5.0	71	6.0	12
	+	0.6	5.3	76	7.0	13
	+	0.7	6.3	90	7.0	11
	+	0.8	T	T	T	T
	+	0.9	T	T	T	T

* T, toxic.

Table 45

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

DL-cis/trans CHRYSANTHEMIC ACID

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		8.6	100	4.6	4.7
	+		9.1	100	11.0	12
Positive control 1,2,3,4-Diepoxybutane	-	0.025	7.0	81	888.0	1,300
	+	0.025	7.5	82	757.5	1,000
DL-cis/trans chrysanthemic acid	-	0.600	9.0	105	7.0	7.8
	-	0.700	9.7	113	17.0	78
	-	0.725	9.2	107	9.0	9.8
	-	0.750	7.9	92	19.0	24
	-	0.775	9.6	112	11.0	11
	-	0.800	10.7	124	15.0	14.0
	+	0.600	8.7	96	8.0	9.2
	+	0.700	7.2	79	10.0	14.0
	+	0.725	7.5	82	15.0	20.0
	+	0.750	8.4	92	14.0	17
	+	0.775	7.5	82	4.0	5.3
	+	0.800	T*	T	T	T

* T, toxic.

Table 46

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
DITHANE M-22

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		8.6	100	4.0	4.7
	+		9.1	100	11.0	12
Positive control 1,2,3,4-Diepoxybutane	-	0.025	7.0	81	888.0	1300
	+	0.025	7.5	82	757.5	1000
Dithane M-22	-	0.0010	5.8	67	8.0	14
	-	0.0025	6.2	72	8.0	13
	-	0.0050	3.8	44	12.0	32
	-	0.0075	4.9	57	9.0	18
	-	0.0100	8.5	99	9.0	11
	-	0.0250	0.3	3	5.0	170
	-	0.0500	0.2	2	1.3	65
	+	0.0010	9.0	98	9.0	10
	+	0.0025	9.6	105	15.0	16
	+	0.0050	10.5	115	9.0	8.6
	+	0.0075	5.9	65	7.0	12
	+	0.0100	7.8	86	12.0	15
	+	0.0250	6.5	71	8.0	12
	+	0.0500	2.6	29	5.0	19

Table 47

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

DITHANE M-22

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		8.9	100	4.5	5.1
	+		7.1	100	5.0	7.0
Positive control 1,2,3,4-Diepoxybutane	-	0.025	10.4	117	530.0	510
	+	0.025	6.7	94	767.5	1100
Dithane M-22	-	0.0010	9.2	103	8.0	8.7
	-	0.0025	9.3	104	5.0	5.4
	-	0.0050	2.1	24	7.0	33
	-	0.0075	5.9	66	11.0	19
	-	0.0100	T*	T	T	T
	+	0.0010	10.9	154	12.0	11.0
	+	0.0025	11.8	166	1.0	0.9
	+	0.0050	8.6	121	9.0	11
	+	0.0075	10.3	145	11.0	11
	+	0.0100	6.3	89	7.0	11

* T, toxic.

Table 48

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

DITHANE M-45

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		8.6	100	4.0	4.7
	+		9.1	100	11.0	12
Positive control 1,2,3,4-Diepoxybutane	-	0.025	7.0	81	888.0	1300
	+	0.025	7.5	82	757.5	1000
Dithane M-45	-	0.0010	6.9	80	14.0	20
	-	0.0025	7.6	88	15.0	20
	-	0.0050	2.5	29	18.0	72.0
	-	0.0075	1.5	17	13.0	87
	-	0.0100	T*	T	T	T
	+	0.0010	10.7	118	11.0	10
	+	0.0025	9.2	101	9.0	9.8
	+	0.0050	8.6	95	14.0	16
	+	0.0075	8.7	96	20.0	23.0
	+	0.0100	3.6	40	22.0	61

* T, toxic.

Table 49

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

DITHANE M-45

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		8.9	100	4.5	5.1
	+		7.1	100	5.0	7.0
Positive control 1,2,3,4-Diepoxybutane	-	0.025	10.4	117	530.0	510
	+	0.025	6.7	94	767.5	1100
Dithane M-45	-	0.0010	10.2	115	6.0	5.9
	-	0.0025	9.4	106	14.0	15
	-	0.0050	6.5	73	8.0	12
	-	0.0075	3.7	42	14.0	38
	-	0.0100	2.4	27	7.0	30
	+	0.0010	10.0	141	8.0	8.0
	+	0.0025	11.5	162	6.3	5.5
	+	0.0050	6.9	97	17.0	25
	+	0.0075	7.0	99	15.0	21
	+	0.0100	6.1	86	13.0	21

Table 50

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

ETHYLCHRYSANTHEMATE

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		8.9	100	4.5	5.1
	+		7.1	100	5.0	7.0
Positive control 1,2,3,4-Diepoxybutane	-	0.025	10.4	117	530.0	510
	+	0.025	6.7	94	767.5	1120
Ethylchrysanthemate	-	0.50	8.5	96	16.0	19
	-	0.75	6.9	78	13.0	19
	-	1.00	7.6	85	11.0	14
	-	2.00	7.0	79	14.0	20.0
	-	3.00	7.1	80	20.0	28
	-	4.00	6.6	74	18.0	27
	+	1.00	6.1	86	21.0	34
	+	2.00	6.5	92	12.0	18
	+	3.00	6.3	89	15.0	24
	+	4.00	7.9	111	20.0	25

Table 51

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

ETHYLCHRYSANTHEMATE

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.4	100	4.5	7.0
	+		6.3	100	5.0	7.9
Positive control 1,2,3,4-Diepoxybutane	-	0.025	6.9	108	687.0	1100
	+	0.025	6.1	97	470.0	770
Ethylchrysanthemate	-	0.50	5.2	81	35.0	67
	-	0.75	5.5	86	29.0	53
	-	1.00	4.9	77	44.0	90
	-	2.00	5.1	80	15.0	29
	-	4.00	2.3	36	9.0	39
	+	1.00	4.2	67	11.0	26
	+	2.00	6.1	97	33.0	54
	+	3.00	4.8	76	35.0	73
	+	4.00	6.0	95	18.0	30
	+	5.00	5.7	90	14.0	25

Table 52

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

MANZATE-D

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.5	100	4.5	6.9
	+		6.5	100	1.5	2.3
Positive control	-		5.9	91	392.5	670
1,2,3,4-Diepoxybutane	+		5.5	85	417.5	760
Manzate-D	-	0.001	5.8	89	10.0	17
	-	0.002	6.3	97	20.0	32
	-	0.003	3.2	49	9.0	28
	-	0.004	2.1	32	7.0	33
	-	0.005	T*	T	T	T
	+	0.006	5.6	86	11.0	20
	+	0.008	5.5	85	8.0	15
	+	0.010	3.7	57	15.0	41
	+	0.015	2.7	42	2.0	7.4
	+	0.020	T	T	T	T

* T, toxic.

Table 53

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

MANZATE-D

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.4	100	4.5	7.0
	+		6.3	100	5.0	7.9
Positive control 1,2,3,4-Diepoxybutane	-		6.9	108	687.0	1000
	+		6.1	97	470.0	770
Manzate-D	-	0.0010	6.5	102	5.0	7.7
	-	0.0020	5.2	81	12.0	23
	-	0.0030	5.7	89	10.0	18
	-	0.0040	4.7	73	13.0	28
	-	0.0050	4.7	73	10.0	21
	+	0.0080	4.6	73	11.0	24
	+	0.0090	6.4	102	15.0	23
	+	0.0100	5.3	84	5.0	9.4
	+	0.0125	5.0	79	9.0	18
	+	0.0150	5.2	83	6.0	12
	+	0.0175	6.0	95	9.0	15
	+	0.0200	6.0	95	6.0	10

Table 54

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

MANZATE 200

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		8.9	100	4.5	5.1
	+		7.1	100	5.0	7.0
Positive control 1,2,3,4-Diepoxybutane	-	0.025	10.4	117	530.0	510
	+	0.025	6.7	94	767.5	1100
Manzate 200	-	0.0010	10.0	112	7.0	7.0
	-	0.0025	8.4	94	10.0	12
	-	0.0050	7.9	89	12.0	15
	-	0.0075	5.9	66	20.0	34
	-	0.0100	5.2	58	20.0	38
	+	0.0010	10.0	141	7.0	7.0
	+	0.0025	8.9	125	17.0	19
	+	0.0050	9.3	131	11.0	12
	+	0.0075	8.6	121	9.0	10
	+	0.0100	6.7	94	19.0	28

Table 55

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

MANZATE 200

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.4	100	4.5	7.0
	+		6.3	100	5.0	7.9
Positive control	-		6.9	108	687.0	1000
1,2,3,4-Diepoxybutane	+		6.1	97	470.0	770
Manzate 200	-	0.0010	7.4	116	7.0	9.5
	-	0.0020	5.8	91	3.0	5.2
	-	0.0030	4.7	73	10.0	21
	-	0.0040	6.3	98	12.0	19
	-	0.0050	6.3	98	13.0	21
	-	0.0060	5.4	84	7.0	13
	-	0.0070	4.2	66	10.0	24
	+	0.0050	6.8	108	12.0	18
	+	0.0075	5.2	83	6.0	12
	+	0.0100	4.9	78	10.0	20
	+	0.0125	6.5	103	17.0	26
	+	0.0150	4.7	75	10.0	21

Table 56

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

NRDC-149

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.4	100	7	11
	+		6.6	100	3	4.5
Positive control 1,2,3,4-Diepoxybutane	+	0.04	4.3	65	1347	3160
NRDC-149	-	0.1	5.9	92	8	14
	-	0.5	5.5	86	4	7.3
	-	1.0	4.9	77	6	12
	-	5.0	6.4	100	5	7.8
	+	0.1	5.1	77	6	12.0
	+	0.5	3.4	52	10	29.0
	+	1.0	4.3	65	9	21
	+	5.0	5.9	89	1	1.7

Table 57

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
NRCD-149

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		7.9	100	6.5	8.2
	+		8.5	100	5.5	6.5
Positive control 1,2,3,4-Diepoxybutane	-	0.025	8.9	113	563	630
	+	0.025	7.6	89	630	830
NRDC-149	-	1.0	8.2	104	5	6.1
	-	2.0	7.6	96	6	7.9
	-	4.0	7.3	92	9	12
	-	5.0	7.5	95	5	6.7
	+	1.0	8.1	95	4	4.[illegible]
	+	2.0	8.6	101	5	5.8
	+	4.0	7.4	87	5	6.8
	+	5.0	8.6	101	6	7.0

Table 58

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

PERMETHRIN

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.4	100	7	11
	+		6.6	100	3	4.5
Positive control 1,2,3,4-Diepoxybutane	+	0.04	4.3	65	1347	3100
Permethrin	-	0.1	8.4	131	3	3.6
	-	0.5	8.0	125	4	5.0
	-	1.0	5.9	92	1	1.7
	-	5.0	4.1	64	2	4.9
	+	0.1	7.0	106	3	4.3
	+	0.5	7.6	115	4	5.3
	+	1.0	7.2	109	4	5.6
	+	5.0	4.6	70	4	8.7

Table 59

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

PERMETHRIN

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		7.9	100	6.5	8.2
	+		8.5	100	5.5	6.5
Positive control 1,2,3,4-Diepoxybutane	-	0.025	8.9	113	563	630
	+	0.025	7.6	89	630	830
Permethrin	-	1.0	6.7	85	3	4.5
	-	2.0	6.8	86	7	10
	-	4.0	8.0	101	8	10.0
	-	5.0	C*	C	C	C
	+	1.0	6.5	76	4	6.2
	+	2.0	6.0	71	6	10
	+	4.0	7.0	82	3	4.3
	+	5.0	6.2	73	6	9.7

* C, contaminated.

Table 60

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

POLYRAM

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		7.9	100	6.5	8.2
	+		8.5	100	5.5	6.2
Positive control 1,2,3,4-Diepoxybutane	-	0.025	8.9	113	563.0	630
	+	0.025	7.6	89	630.0	830
Polyram	-	0.0005	9.0	114	4.0	4.4
	-	0.0001	8.3	105	3.0	3.6
	-	0.0010	8.5	108	6.0	7.1
	-	0.0050	2.1	27	4.0	19
	+	0.0005	9.5	112	9.0	9.5
	+	0.0001	8.5	100	5.0	5.9
	+	0.0010	8.5	100	9.0	11
	+	0.0050	8.4	99	5.0	6.0

Table 61

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

POLYRAM

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.6	100	6.5	12
	+		7.0	100	4.5	6.4
Positive control 1,2,3,4-Diepoxybutane	-	0.025	8.4	150	485.0	580
	+	0.025	7.7	110	650.0	840
Polyram	-	0.004	6.5	116	11.0	17
	-	0.005	2.6	46	5.0	19
	-	0.006	1.7	30	1.0	5.9
	-	0.007	1.3	23	< 1.0	< 7.7
	-	0.008	T*	T	T	T
	+	0.004	8.4	120	8.0	9.5
	+	0.005	8.6	123	8.0	9.3
	+	0.006	7.8	111	7.0	9.0
	+	0.007	6.2	89	4.0	6.5
	+	0.008	8.5	121	8.0	9.4

* T, toxic.

Table 62

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

RESMETHRIN

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		5.0	100	2	4.0
	+		4.7	100	3	6.4
Positive control 1,2,3,4-Diepoxybutane	-	0.04	5.8	116	630	1100
	+	0.04	4.1	87	435	1100
Resmethrin	-	0.1	6.5	130	1	1.5
	-	0.5	3.9	78	1	2.6
	-	1.0	4.4	88	2	4.6
	-	5.0	4.4	88	2	4.6
	+	0.1	5.3	113	2	3.8
	+	0.5	3.6	77	3	8.3
	+	1.0	4.2	89	2	4.8
	+	5.0	4.3	91	2	4.7

Table 63

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

RESMETHRIN

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		7.0	100	6	8.6
	+		6.5	100	7	11
Positive control 1,2,3,4-Diepoxybutane	-	0.04	7.5	107	738	980
	+	0.04	7.1	109	778	1100
Resmethrin	-	1.0	3.6	51	4	11
	-	2.0	3.2	46	1	3.1
	-	4.0	4.4	63	4	9.1
	-	5.0	3.5	50	3	8.6
	+	1.0	3.3	51	4	12
	+	2.0	4.0	62	1	2.5
	+	4.0	2.6	40	3	12
	+	5.0	4.1	63	3	7.3

Table 64

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

SUMITHRIN

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.4	100	7	11
	+		6.6	100	3	4.5
Positive control 1,2,3,4-Diepoxybutane	+	0.04	4.3	65	1347	3100
Sumithrin	-	0.1	5.2	81	4	7.7
	-	0.5	4.6	72	4	8.7
	-	1.0	4.4	69	3	6.8
	-	5.0	4.1	64	4	9.8
	+	0.1	4.2	64	4	9.5
	+	0.5	4.3	65	5	12
	+	1.0	3.6	55	2	5.6
	+	5.0	6.5	98	2	3.1

Table 65

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

SUMITHRIN

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		9.5	100	6.5	6.8
	+		9.7	100	9.5	9.8
Positive control 1,2,3,4-Diepoxybutane	-	0.025	8.9	94	995	1100
	+	0.025	6.9	71	790	1100
Sumithrin	-	1.0	9.5	100	7.0	7.4
	-	2.0	9.5	100	10.0	11
	-	4.0	9.6	101	5.0	5.2
	-	5.0	8.5	89	3.0	3.5
	+	1.0	8.5	88	7.0	8.2
	+	2.0	10.9	112	6.0	5.5
	+	4.0	9.1	94	5.0	5.5
	+	5.0	6.4	66	8.0	13

Table 66

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

ZINEB

Experiment 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		8.9	100	4.5	5.1
	+		7.1	100	5.0	7.0
Positive control 1,2,3,4-Diepoxybutane	-	0.025	10.4	117	530.0	510
	+	0.025	6.7	94	767.5	1100
Zineb	-	0.001	10.6	119	12.0	11
	-	0.005	3.0	34	14.0	47
	-	0.010	2.2	25	5.0	23
	-	0.050	T*	T	T	T
	+	0.005	C†	C	C	C
	+	0.010	8.0	113	6.0	7.5
	+	0.050	5.6	79	42.0	75
	+	0.100	2.2	31	9.0	41

* T, toxic.
† C, contaminated.

Table 67

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

ZINEB

Experiment 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative control	-		6.4	100	4.5	7.0
	+		6.3	100	5.0	7.9
Positive control 1,2,3,4-Diepoxybutane	-		6.9	108	687.0	1000
	+		6.1	97	470.0	770
Zineb	-	0.0008	8.6	134	3.0	3.5
	-	0.0010	6.8	106	7.0	10
	-	0.0020	8.1	127	11.0	14
	-	0.0030	7.0	109	11.0	16
	-	0.0040	5.7	89	8.0	14
	-	0.0050	5.4	84	14.0	26
	-	0.0060	4.9	77	18.0	37
	-	0.0070	3.8	59	16.0	42
	+	0.0300	6.8	108	10.0	15
	+	0.0400	5.3	84	7.0	13
	+	0.0500	3.9	62	5.0	13
	+	0.0600	3.1	49	10.0	32
	+	0.0700	4.9	78	4.0	8.2
	+	0.0800	2.1	33	7.0	33
	+	0.0900	1.7	27	1.0	5.9
	+	0.1000	1.4	22	4.0	29

Table 68

DIFFERENTIAL TOXICITY TO DNA REPAIR-PROFICIENT AND REPAIR-DEFICIENT BACTERIA

Compound	Milligrams of Compound Applied to Disc	Average Diameter of Zone of Inhibition* (in mm, disc = 6 mm)			
		E. coli		B. subtilis	
		W3110	p3478	H17	M45
Negative control (ampicillin)	0.1	16	16	20	21
Positive controls					
1-Phenyl-3,3-dimethyltriazene	1.0	36	56	51	63
Methylmethanesulfonate	1.0	26	33	17	43
Bioallethrin	10	6	6	8	9
	20	6	6	11	12
Biphenyl	1	6	6	6	6
	5	6	6	6	6
Chlordimeform	1	6	7	6	6
	5	6	6	8	8
DL-cis/trans chrysanthemic acid	0.5	6	6	14	16
	1.0	7	7	14	17
	5.0	9	9	10	13
Dithane M-22	0.05	23	20	19	19
	0.1	23	21	20	21
	0.5	25	24	25	28
	1.0	30	31	26	27
Dithane M-45	0.05	21	21	19	20
	0.1	21	20	20	20
	0.5	23	21	25	26
	1.0	29	28	26	24
Ethylchrysanthemate	10	6	6	9	10
	20	8	9	12	13

*Average of at least three plates.

Table 68 (Concluded)

Compound	Milligrams of Compound Applied to Disc	Average Diameter of Zone of Inhibition* (in mm, disc = 6 mm)			
		E. coli		B. subtilis	
		W3110	p3478	H17	M45
Manzate-D	0.1	18	18	21	20
	0.5	22	23	29	27
	1.0	29	29	25	24
Manzate 200	0.1	12	12	16	17
	0.5	20	20	19	20
	1.0	29	26	26	26
	5.0	29	27	25	23
NRDC-149	1	6	6	6	6
	10	6	6	6	6
Permethrin	1	6	6	6	6
	5	6	6	6	6
Polyram	0.1	13	14	16	17
	0.5	14	14	16	18
	1.0	22	21	22	23
	5.0	24	24	26	27
Resmethrin	1	6	6	6	6
	5	6	6	6	6
Sumithrin	10	6	6	6	6
	20	6	6	6	6
Zineb	1	16	15	17	16
	5	13	14	12	14

*Average of at least three plates.

Table 69

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF BIOALLETHRIN
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	Bioallethrin (µg/ml)					4NQO (M)
	0*	2	20	200†	2000†	10^{-5}
1	80	59	102	61	63	1450
2	55	104	97	46	52	1395
3	78	79	102	54	36	1039
4	85	62	77	62	28	415
Mean	75	76	95	56	45	1075
SD	13	21	12	7	16	476
SE	7	10	6	4	8	238

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 200 and 2000 µg/ml.

Table 70

UNSCHEDULED DNA SYNTHESIS ASSAY OF BIOALLETHRIN
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Bioallethrin (µg/ml)						4NQO (M)
	0*	6.3	12.5	25	50†	100†	10^{-5}
1	41	53	60	70	61	45	1583
2	45	39	62	83	68	66	1401
3	45	66	66	78	66	59	1353
4	37	38	69	69	64	53	1516
5	57	67	65	83	68	65	1878
6	56	89	72	71	62	47	1454
Mean	47	59	66	76	65	56	1531
SD	8	20	4	7	3	9	189
SE	3	8	2	3	1	4	77

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 50 and 100 µg/ml.

Table 71

SECOND UNSCHEDULED DNA SYNTHESIS ASSAY OF BIOALLETHRIN
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Bioallethrin (µg/ml) 0*	11.11	16.7	25	37.5	56.25	4NQO (M) 10^{-5}
1	27	50	63	52	52	58	1553
2	46	59	85	48	44	52	1614
3	70	84	73	60	67	66	1861
4	65	72	59	60	60	47	1731
5	62	46	56	77	65	64	1565
6	63	58	66	50	57	59	1607
Mean	56	62	67	58	58	58	1655
SD	16	14	10	11	8	7	119
SE	6	6	4	4	3	3	48

*Negative control and compound solvent, 0.5% DMSO.

Table 72

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF BIOALLETHRIN
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Bioallethrin (µg/ml) 0*	4	40	400†	4000†	DMN (M) 5×10^{-2}
1	116	85	86	86	73	452
2	81	79	97	58	54	444
3	117	94	54	113	85	466
4	63	64	60	85	76	382
Mean	94	81	74	85	72	436
SD	27	12	20	22	13	37
SE	13	6	10	11	7	19

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 400 and 4000 µg/ml.

Table 73

UNSCHEDULED DNA SYNTHESIS ASSAY OF BIOALLETHRIN
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Bioallethrin (µg/ml)						
Sample	0*	313†	625†	1250†	2500†	5000†	DMN (M) 5×10^{-2}
1	73	62	72	86	56	39	290
2	58	58	56	62	80	60	382
3	49	34	34	81	56	48	342
4	56	48	53	53	65	84	358
5	80	40	39	56	29	68	280
6	100	47	50	53	65	50	150
Mean	69	48	51	65	59	58	300
SD	19	10	14	15	17	16	84
SE	8	4	6	6	7	6	34

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at all concentrations.

Table 74

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF BIPHENYL
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	Biphenyl (µg/ml)					4NQO (M)
	0*	2	20	200†	2000†	10^{-5}
1	150	84	97	71	96	1192
2	117	93	84	62	64	969
3	99	92	96	98	104	1254
4	93	97	78	88	123	434
Mean	114	91	89	80	97	962
SD	26	5	10	16	24	373
SE	13	3	5	8	12	186

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 200 and 2000 µg/ml.

Table 75

UNSCHEDULED DNA SYNTHESIS ASSAY OF BIPHENYL
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Biphenyl (µg/ml) 0*	125†	250†	500†	1000†	2000†	4NQO (M) 10^{-5}
1	78	63	46	68	85	107	2022
2	48	36	60	50	89	182	2050
3	66	97	59	35	70	87	2093
4	91	60	55	45	74	78	2083
5	70	73	37	58	86	85	2434
6	59	55	95	44	55	90	1518
Mean	69	64	59	50	77	105	2033
SD	15	23	20	12	13	39	294
SE	6	9	8	5	5	16	120

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at all concentrations.

Table 76

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF BIPHENYL
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested					
	Biphenyl (µg/ml)					DMN (M)
Sample	0*	4	40	400†	4000†	5×10^{-2}
1	119	209	92	107	109	682
2	112	130	142	70	164	670
3	116	95	93	118	213	717
4	62	135	84	74	167	336
Mean	102	142	103	92	163	601
SD	27	48	27	24	43	178
SE	13	24	13	12	21	89

*Negative control and compound solvent, 1.0% DMSO.

†Precipitates observed at 400 and 4000 µg/ml.

Table 77

UNSCHEDULED DNA SYNTHESIS ASSAY OF BIPHENYL WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Biphenyl (µg/ml) 0*	250†	500†	1000†	2000†	4000†	DMN (M) 5×10^{-2}
1	56	43	59	56	54	40	227
2	67	52	47	61	62	64	245
3	72	64	52	62	71	54	266
4	52	54	60	60	62	68	217
5	65	50	62	55	51	68	223
6	82	56	65	68	73	57	219
Mean	66	53	58	60	62	58	233
SD	11	7	7	5	9	11	19
SE	4	3	3	2	4	4	8

*Negative control and compound solvent, 1.0% DMSO.

†Precipitates observed at all concentrations.

Table 78

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF CHLORDIMEFORM
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	Chlordimeform (µg/ml)					4NQO (M) 10^{-5}
	0*	2	20	200	2000†	
1	80	42	61	68	7	1450
2	55	45	67	78	13	1395
3	78	57	93	72	6	1039
4	85	64	71	46	8	415
Mean	75	52	73	66	9	1075
SD	13	11	14	14	3	476
SE	7	5	7	7	1	238

*Negative control and compound solvent, 0.5% DMSO.
†Precipitate observed at 2000 µg/ml.

Table 79

UNSCHEDULED DNA SYNTHESIS ASSAY OF CHLORDIMEFORM
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Chlordimeform (µg/ml) 0*	62.5	125	250	500	1000†	4NQO (M) 10^{-5}
1	78	40	65	36	109	41	2022
2	48	50	49	50	64	41	2050
3	66	70	97	67	78	29	2093
4	91	38	64	48	39	14	2083
5	70	62	49	35	36	36	2434
6	59	38	73	64	52	27	1518
Mean	69	50	66	50	63	31	2033
SD	15	14	18	14	28	10	294
SE	6	6	7	6	11	4	120

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at 1000 µg/ml.

Table 80

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF CHLORDIMEFORM
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested					
	Chlordimeform (µg/ml)					DMN (M)
Sample	0*	4	40	400†	5000†	5×10^{-2}
1	119	118	108	102	192	682
2	112	104	131	104	106	670
3	116	101	99	119	220	717
4	62	129	125	88	221	336
Mean	102	113	116	103	184	601
SD	27	13	15	13	54	178
SE	13	7	7	6	27	89

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 400 and 5000 µg/ml.

Table 81

UNSCHEDULED DNA SYNTHESIS ASSAY OF CHLORDIMEFORM
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Chlordimeform (µg/ml) 0*	313	625	1250†	2500†	5000†	DMN (M) 5 x 10^{-2}
1	69	65	61	60	68	25	417
2	58	66	70	68	68	28	379
3	58	59	77	65	58	36	355'
4	58	62	61	65	64	24	397
5	62	61	53	72	77	35	432
6	59	70	50	75	72	31	414
Mean	61	64	62	68	68	30	399
SD	4	4	10	5	7	5	28
SE	2	2	4	2	3	2	11

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 1250, 2500 and 5000 µg/ml.

Table 82

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF
DL-cis/trans CHRYSANTHEMIC ACID
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	DL-cis/trans Chrysanthemic acid (µg/ml)					4NQO (M)
	0*	2	20	200	2000†	10^{-5}
1	103	184	102	116	128	1610
2	94	88	43	146	106	976
3	43	81	57	105	102	2012
4	66	61	65	69	82	1890
Mean	77	103	67	109	105	1622
SD	28	55	25	32	19	462
SE	14	27	13	16	9	231

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at 2000 µg/ml.

Table 83

UNSCHEDULED DNA SYNTHESIS ASSAY OF DL-*cis*/*trans* CHRYSANTHEMIC ACID
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: DL cis/trans Chrysanthemic Acid (µg/ml) 0*	250	500	1000	2000†	4000†	4NQO (M) 10^{-5}
1	48	45	53	77	98	69	1676
2	62	52	46	41	43	47	1763
3	87	55	84	65	64	44	1894
4	50	65	96	104	46	47	1244
5	58	100	70	73	38	64	1684
6	103	44	76	54	46	58	1405
Mean	68	60	71	69	56	55	1611
SD	22	21	19	22	22	10	241
SE	9	9	8	9	9	4	98

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 2000 and 4000 µg/ml.

Table 84

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF DL-cis/trans CHRYSANTHEMIC ACID WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: DL cis/trans chrysanthemic acid (µg/ml) 0*	4	40	400	4000	DMN (M) 5×10^{-2}
1	75	55	96	112	77	567
2	81	87	61	89	78	596
3	90	83	68	99	118	492
4	72	86	61	74	84	506
Mean	80	78	71	93	89	540
SD	8	15	17	16	20	49
SE	4	8	8	8	10	25

*Negative control and compound solvent, 0.5% DMSO.

Table 85

UNSCHEDULED DNA SYNTHESIS ASSAY OF DL-cis/trans CHRYSANTHEMIC ACID WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: DL cis/trans Chrysanthemic Acid (µg/ml) 0*	313	625	1250	2500	5000	DMN (M) 5×10^{-2}
1	73	65	66	86	87	64	290
2	58	74	66	72	68	48	382
3	49	83	72	67	60	58	342
4	56	63	83	92	72	55	358
5	80	83	67	80	68	103	280
6	100	45	44	70	73	67	150
Mean	69	69	66	78	71	66	300
SD	19	15	13	10	9	19	84
SE	8	6	5	4	4	8	34

*Negative control and compound solvent, 0.5% DMSO.

Table 86

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF DITHANE M-22
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Dithane M-22 (µg/ml) 0*	2	20	200†	2000†	4NQO (M) 10^{-5}
1	150	108	26	6	0	1192
2	117	75	35	6	3	969
3	99	143	20	4	4	1254
4	93	91	23	3	3	434
Mean	114	104	26	5	3	962
SD	26	29	6	1	2	373
SE	13	15	3	0.5	1	186

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 200 and 2000 µg/ml.

Table 87

UNSCHEDULED DNA SYNTHESIS ASSAY OF DITHANE M-22
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Dithane M-22 (µg/ml) 0*	0.8	2.0	5.0	12.5	31.3	4NQO (M) 10^{-5}
1	193	191	204	166	8	--†	2352
2	182	213	163	147	15	14	2476
3	200	202	179	127	12	15	2117
4	--†	270	189	80	5	22	2143
5	281	146	162	217	19	16	1605
6	337	162	293	245	16	23	2157
Mean	239	197	198	164	13	18	2142
SD	68	43	49	60	5	4	298
SE	30	18	20	25	2	2	122

*Negative control and compound solvent, 0.5% DMSO.

†Sample lost.

Table 88

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF DITHANE M-22
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested					
	Dithane M-22 (µg/ml)					DMN (M)
Sample	0*	2	20	200†	2000†	5×10^{-2}
1	65	89	68	70	69	509
2	66	94	89	56	67	492
3	56	44	92	81	56	485
4	84	73	86	88	76	302
Mean	68	75	83	74	67	447
SD	12	23	10	14	7	97
SE	6	11	5	7	4	49

*Negative control and compound solvent, 1.0% DMSO.

†Precipitates observed at 200 and 2000 µg/ml.

Table 89

UNSCHEDULED DNA SYNTHESIS ASSAY OF DITHANE M-22
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Dithane M-22 (µg/ml) 0*	250†	500†	1000†	2000†	4000†	DMN (M) 5×10^{-2}
1	52	49	52	62	48	11	448
2	60	71	65	85	49	74	363
3	55	63	63	72	56	44	122
4	51	68	55	65	58	19	401
5	66	70	64	48	38	35	409
6	50	47	73	64	24	9	426
Mean	56	62	62	66	46	32	361
SD	6	11	8	12	13	25	121
SE	3	5	3	5	5	10	49

*Negative control and compound solvent, 1.0% DMSO.

†Precipitates observed at all concentrations.

Table 90

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF DITHANE M-45
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	Dithane M-45 (µg/ml)					4NQO (M)
	0*	2	20	200†	2000†	10^{-5}
1	150	146	22	2	2	1192
2	117	93	13	2	3	969
3	99	114	40	1	2	1254
4	93	82	11	1	2	434
Mean	114	109	22	2	2	962
SD	26	28	13	1	1	373
SE	13	14	6	0.5	0.5	186

*Negative control and compound solvent, 0.5% DMSO.
†Precipitates observed at 200 and 2000 µg/ml.

Table 91

UNSCHEDULED DNA SYNTHESIS ASSAY OF DITHANE M-45
(dpm/μg DNA)

Sample	Concentration of Compounds Tested: Dithane M-45 (μg/ml) 0*	0.8	2.0	5.0	12.5	31.3	4NQO (M) 10^{-5}
1	193	166	187	152	22	14	2352
2	182	189	251	142	24	3	2476
3	200	193	263	130	21	2	2117
4	--†	107	184	151	21	9	2143
5	281	167	308	147	17	7	1605
6	337	169	180	162	11	†	2157
Mean	239	165	229	147	19	7	2142
SD	68	31	53	11	4	5	298
SE	30	13	22	4	2	2	122

*Negative control and compound solvent, 0.5% DMSO.

†Sample lost.

Table 92

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF DITHANE M-45
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	Dithane M-45 (µg/ml)					DMN (M)
	0*	2	20	200†	2000†	5×10^{-2}
1	65	81	80	70	85	509
2	66	46	72	72	58	492
3	56	76	73	62	57	485
4	84	72	106	65	47	302
Mean	68	69	83	67	62	447
SD	12	15	16	5	16	97
SE	6	8	8	2	8	49

*Negative control and compound solvent, 1.0% DMSO.

†Precipitates observed at 200 and 2000 µg/ml.

Table 93

UNSCHEDULED DNA SYNTHESIS ASSAY OF DITHANE M-45
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Dithane M-45 (µg/ml) 0*	250†	500†	1000†	2000†	4000†	DMN (M) 5×10^{-2}
1	52	43	54	61	52	3	448
2	60	72	44	61	35	7	363
3	55	65	51	59	28	3	122
4	51	53	84	54	57	2	401
5	66	59	54	39	31	3	409
6	50	47	37	60	41	9	426
Mean	56	56	54	56	41	5	361
SD	6	11	16	9	11	3	121
SE	3	5	7	4	5	1	49

*Negative control and compound solvent, 1.0% DMSO.

†Precipitates observed at all concentrations.

Table 94

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF ETHYLCHRYSANTHEMATE
(dpm/µg DNA)

	Concentration of Compounds Tested					
	Ethylchrysanthemate (µg/ml)					4NQO (M)
Sample	0*	2	20	200	2000†	10^{-5}
1	80	74	82	21	25	1450
2	55	71	67	21	23	1395
3	78	59	78	25	18	1039
4	85	46	70	28	10	415
Mean	75	62	74	24	19	1075
SD	13	13	7	3	7	476
SE	7	6	3	2	3	238

*Negative control and compound solvent, 0.5% DMSO.

†Droplets of compound observed at 2000 µg/ml.

Table 95

UNSCHEDULED DNA SYNTHESIS ASSAY OF ETHYLCHRYSANTHEMATE
(dpm/µg DNA)

Sample	Concentration of Compounds Tested						
	Ethylchrysanthemate (µg/ml)						4NQO (M)
	0*	6.3	12.5	25	50	100	10^{-5}
1	41	40	43	59	73	77	1583
2	45	47	33	71	75	76	1401
3	45	39	53	85	72	61	1353
4	37	43	53	99	54	57	1516
5	57	50	56	60	78	90	1878
6	56	95	59	61	71	76	1454
Mean	47	52	50	73	70	73	1531
SD	8	21	10	16	8	12	189
SE	3	9	4	7	3	5	77

*Negative control and compound solvent, 0.5% DMSO.

Table 96

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF ETHYLCHRYSANTHEMATE WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Ethylchrysanthemate (µg/ml) 0*	4	40	400	4000	DMN (M) 5×10^{-2}
1	116	118	108	60	28	452
2	81	108	65	--†	--†	444
3	117	61	137	136	33	466
4	63	98	74	57	20	382
Mean	94	96	96	84	27	436
SD	27	25	33	45	7	37
SE	13	12	16	26	4	19

*Negative control and compound solvent, 0.5% DMSO.

†Sample lost.

Table 97

UNSCHEDULED DNA SYNTHESIS ASSAY OF ETHYLCHRYSANTHEMATE
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Ethylchrysanthemate (µg/ml)						DMN (M)
Sample	0*	250	500	1000†	2000†	4000†	5×10^{-2}
1	58	49	60	51	22	37	526
2	74	51	65	71	43	36	427
3	60	78	69	67	30	40	329
4	81	59	68	64	34	29	350
5	60	51	50	57	37	27	449
6	59	66	64	36	40	22	--‡
Mean	65	59	63	58	34	32	416
SD	10	12	7	13	7	7	79
SE	4	5	3	5	3	3	35

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 1000, 2000, and 4000 µg/ml.

‡Sample lost.

Table 98

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF MANZATE-D
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	Manzate-D (µg/ml)					4NQO (M)
	0*	2	20	200†	2000†	10^{-5}
1	150	103	52	4	0	1192
2	117	132	48	6	2	969
3	99	74	41	7	2	1254
4	93	91	24	3	0	434
Mean	114	100	41	5	1	962
SD	26	24	12	2	1	373
SE	13	12	6	1	0.5	186

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 200 and 2000 µg/ml.

Table 99

UNSCHEDULED DNA SYNTHESIS ASSAY OF MANZATE-D
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Manzate-D (µg/ml) 0*	0.8	2.0	5.0	12.5	31.3†	4NQO (M) 10^{-5}
1	--‡	57	58	89	36	23	1361
2	57	68	60	81	29	21	1509
3	73	68	89	104	32	20	1418
4	58	65	68	75	38	21	1432
5	50	92	68	80	42	15	1745
6	60	47	53	138	30	28	1419
Mean	60	66	66	94	34	21	1481
SD	8	15	13	23	5	4	138
SE	4	6	5	10	2	2	56

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at 31.3 µg/ml.

‡Sample lost.

Table 100

SECOND UNSCHEDULED DNA SYNTHESIS ASSAY OF MANZATE-D
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Manzate-D (µg/ml)						
Sample	0*	2.22	3.33	5	7.5	11.25	4NQO (M) 10^{-5}
1	50	42	62	87	97	73	1446
2	59	55	73	72	100	72	1684
3	58	57	79	74	83	63	1736
4	48	69	76	93	103	70	1575
5	49	64	53	106	101	68	1532
6	54	44	56	126	113	67	1140
Mean	53	55	66	93	99	69	1519
SD	5	11	11	20	10	4	213
SE	2	4	4	8	4	1	87

*Negative control and compound solvent, 0.5% DMSO.

Table 101

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF MANZATE-D
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	Manzate-D (µg/ml)					DMN (M)
	0*	2	20	200†	2000†	5×10^{-2}
1	65	61	62	61	50	509
2	66	61	69	85	66	492
3	56	69	87	62	66	485
4	84	74	63	67	63	302
Mean	68	66	70	69	61	447
SD	12	6	12	11	8	97
SE	6	3	6	6	4	49

*Negative control and compound solvent, 1.0% DMSO.

†Precipitates observed at 200 and 2000 µg/ml.

Table 102

UNSCHEDULED DNA SYNTHESIS ASSAY OF MANZATE-D
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested						
	Manzate-D (µg/ml)						DMN (M)
	0*	125†	250†	500†	1000†	2000†	5×10^{-2}
1	73	68	77	90	62	86	430
2	68	94	75	84	58	67	447
3	66	64	81	81	66	76	480
4	62	66	66	43	56	58	507
5	68	70	69	--‡	84	90	588
6	88	98	66	52	79	77	532
Mean	71	77	72	70	68	76	497
SD	9	15	6	21	11	12	58
SE	4	6	3	10	5	5	24

*Negative control and compound solvent, 1.0% DMSO.

†Precipitates observed at all concentrations.

‡Sample lost.

Table 103

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF MANZATE 200
(dpm/µg DNA)

	Concentration of Compounds Tested					
	Manzate 200 (µg/ml)					4NQO (M)
Sample	0*	2	20	200†	2000†	10^{-5}
1	53	50	43	6	5	1275
2	47	41	54	6	2	1302
3	67	37	33	5	3	1119
4	55	60	42	5	0	1438
Mean	56	47	43	6	3	1284
SD	8	10	9	0.6	2	131
SE	4	5	5	0.3	1	65

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 200 and 2000 µg/ml.

Table 104

UNSCHEDULED DNA SYNTHESIS ASSAY OF MANZATE 200
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Manzate 200 (µg/ml) 0*	0.8	2	5	12.5	31.3†	4NQO (M) 10^{-5}
1	115	73	84	137	28	21	2112
2	120	105	110	122	27	12	2174
3	86	73	96	144	18	12	1857
4	82	103	91	159	28	29	2374
5	104	76	107	187	29	24	971
6	101	58	115	223	38	14	2129
Mean	101	81	100	162	28	19	1936
SD	15	18	12	37	6	7	501
SE	6	8	5	15	3	3	205

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at 31.3 µg/ml.

Table 105

SECOND UNSCHEDULED DNA SYNTHESIS ASSAY OF MANZATE 200
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Manzate 200 (µg/ml) 0*	2.22	3.33	5	7.5	11.25	4NQO (M) 10^{-5}
1	50	49	53	73	91	90	1446
2	59	50	61	71	108	86	1684
3	58	52	74	87	122	89	1736
4	48	59	71	100	112	68	1575
5	49	54	55	82	106	76	1532
6	54	55	56	83	89	87	1140
Mean	53	53	62	83	105	83	1519
SD	5	3	9	10	13	9	213
SE	2	1	4	4	5	4	87

*Negative control and compound solvent, 0.5% DMSO.

Table 106

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF MANZATE 200
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	Manzate 200 (µg/ml)					DMN (M)
	0*	2	20	200†	2000†	5×10^{-2}
1	65	76	55	61	72	509
2	66	69	108	63	47	492
3	56	55	96	64	48	485
4	84	63	63	92	59	302
Mean	68	66	80	70	56	447
SD	12	9	26	15	12	97
SE	6	4	13	7	6	49

*Negative control and compound solvent, 1.0% DMSO.

†Precipitates observed at 200 and 2000 µg/ml.

Table 107

UNSCHEDULED DNA SYNTHESIS ASSAY OF MANZATE 200
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Manzate 200 (µg/ml)						DMN (M)
Sample	0*	125†	250†	500†	1000†	2000†	5×10^{-2}
1	73	83	74	86	86	12	430
2	68	78	78	73	74	16	447
3	66	89	67	77	55	15	480
4	62	67	75	60	65	16	507
5	68	64	65	85	73	28	588
6	88	85	83	101	60	25	532
Mean	71	78	73	80	69	19	497
SD	9	10	7	14	11	6	58
SE	4	4	3	6	5	2	24

*Negative control and compound solvent, 1.0% DMSO.

†Precipitates observed at all concentrations.

Table 108

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF NRDC-149
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	NRDC-149 (µg/ml)					4NQO (M)
	0*	2	20	200†	2000†	10^{-5}
1	103	61	173	87	36	1610
2	94	62	77	55	57	976
3	43	74	77	45	78	2012
4	66	59	68	70	60	1890
Mean	77	64	98	64	58	1622
SD	28	7	50	18	18	462
SE	14	3	25	9	9	231

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 200 and 2000 µg/ml.

Table 109

UNSCHEDULED DNA SYNTHESIS ASSAY OF NRDC-149
(dpm/μg DNA)

Sample	Concentration of Compounds Tested						
	NRDC-149 (μg/ml)						4NQO (M)
	0*	250†	500†	1000†	2000†	4000†	10^{-5}
1	48	80	82	70	41	40	1676
2	62	51	56	63	53	44	1763
3	87	75	66	57	46	37	1894
4	50	77	109	60	33	37	1244
5	58	137	92	122	46	30	1684
6	103	78	81	45	70	41	1405
Mean	68	83	81	70	48	38	1611
SD	22	29	19	27	12	5	241
SE	9	12	8	11	5	2	98

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at all concentrations.

Table 110

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF NRDC-149
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	NRDC-149 (µg/ml)					DMN (M)
	0*	4	40	400†	4000†	5×10^{-2}
1	119	133	106	129	100	682
2	112	132	110	99	110	670
3	116	131	132	89	108	717
4	62	164	140	133	134	336
Mean	107	140	122	113	113	601
SD	27	16	17	22	15	178
SE	13	8	8	11	7	89

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 400 and 4000 µg/ml.

Table 111

UNSCHEDULED DNA SYNTHESIS ASSAY OF NRDC-149 WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Concentration of Compounds Tested						
	NRDC-149 (μg/ml)						DMN (M)
Sample	0*	250†	500†	1000†	2000†	4000†	5×10^{-2}
1	58	68	39	53	56	58	526
2	74	57	48	41	67	62	427
3	60	51	56	52	90	60	329
4	81	69	59	58	61	65	350
5	60	73	83	50	65	56	449
6	59	50	54	59	57	73	--‡
Mean	65	61	57	52	66	63	416
SD	10	10	15	6	12	6	79
SE	4	4	6	3	5	2	35

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at all concentrations.

‡Sample lost.

Table 112

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF PERMETHRIN
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	Permethrin (µg/ml)					4NQO (M)
	0*	2	20	200†	2000†	10^{-5}
1	80	80	80	38	108	1450
2	55	52	90	127	87	1395
3	78	64	85	94	115	1039
4	85	88	106	121	121	415
Mean	75	71	90	95	108	1075
SD	13	16	11	40	14	476
SE	7	8	6	20	7	238

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 200 and 2000 µg/ml.

Table 113

UNSCHEDULED DNA SYNTHESIS ASSAY OF PERMETHRIN
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Permethrin (µg/ml)						4NQO (M)
Sample	0*	250†	500†	1000†	2000†	4000†	10^{-5}
1	‡	72	67	72	81	70	1361
2	57	90	73	88	62	74	1509
3	73	61	59	101	60	82	1418
4	58	57	79	70	63	93	1432
5	50	77	71	63	75	83	1745
6	60	83	69	57	64	62	1419
Mean	60	73	70	75	68	77	1481
SD	8	13	7	16	8	11	138
SE	4	5	3	7	3	4	56

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at all concentrations.

‡Sample lost.

Table 114

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF PERMETHRIN
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	Permethrin (µg/ml)					DMN (M) 5×10^{-2}
	0*	4	40	400†	4000†	
1	119	105	--‡	136	112	682
2	112	134	151	113	161	670
3	116	194	159	103	112	717
4	62	105	128	112	108	336
Mean	102	135	146	116	123	601
SD	27	42	16	14	25	178
SE	13	21	9	7	7	89

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 400 and 4000 µg/ml.

‡Sample lost.

Table 15

UNSCHEDULED DNA SYNTHESIS ASSAY OF PERMETHRIN WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Permethrin (µg/ml)						DMN (M)
Sample	0*	15.6	62.5†	250†	1000†	4000†	5×10^{-2}
1	69	37	46	46	61	60	417
2	58	56	49	51	69	64	379
3	58	58	48	57	53	63	355
4	58	47	59	60	50	65	397
5	62	57	50	49	42	53	432
6	59	53	62	48	70	58	414
Mean	61	51	52	52	57	61	399
SD	4	8	6	5	11	4	28
	2	3	2	2	5	2	11

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 62.5, 250, 1000, and 4000 µg/ml.

Table 116

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF POLYRAM
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	Polyram (µg/ml)					4NQO (M)
	0*	2	20	200	2000†	10^{-5}
1	86	108	30	8	4	1582
2	144	144	26	7	2	1656
3	128	ǂ	28	7	2	1799
4	108	ǂ	28	7	4	1559
Mean	117	126	28	7	3	1649
SD	25	25	2	0.5	1	108
SE	12	18	1	0.25	0.5	54

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at 2000 µg/ml.

ǂSample lost.

Table 117

UNSCHEDULED DNA SYNTHESIS ASSAY OF POLYRAM
(dpm/μg DNA)

Sample	Concentration of Compounds Tested: Polyram (μg/ml) 0*	0.8	2.0	5.0	12.5	31.3	4NQO (M) 10^{-5}
1	96	58	98	47	21	12	1890
2	98	81	124	49	18	16	1954
3	74	110	120	37	13	9	2031
4	89	69	108	48	17	19	2077
5	109	99	89	47	24	14	2219
6	127	124	118	36	19	10	1281
Mean	99	90	110	44	19	13	1909
SD	18	25	14	6	4	4	327
SE	7	10	6	2	2	2	134

*Negative control and compound solvent, 0.5% DMSO.

Table 118

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF POLYRAM
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested					
	Polyram (µg/ml)					DMN (M)
Sample	0*	2	20	200	2000†	5×10^{-2}
1	99	99	106	62	4	368
2	121	118	109	86	3	397
3	120	74	104	36	2	387
4	141	132	107	50	5	381
Mean	120	105	107	59	4	383
SD	17	25	2	21	1	12
SE	9	12	1	10	0.5	6

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at 2000 µg/ml.

Table 119

UNSCHEDULED DNA SYNTHESIS ASSAY OF POLYRAM WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Polyram (µg/ml)						DMN (M)
Sample	0*	12.5	25	50	100†	200†	5×10^{-2}
1	41	56	44	38	40	7	331
2	55	57	62	38	44	6	355
3	58	63	63	55	46	6	349
4	50	51	45	48	42	11	370
5	64	49	44	52	48	8	340
6	92	67	50	63	50	10	409
Mean	60	57	51	49	45	8	359
SD	17	7	9	10	4	2	28
SE	7	3	4	4	2	1	11

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 100 and 200 µg/ml.

Table 120

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF RESMETHRIN
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	Resmethrin (µg/ml)					4NQO (M)
	0*	2	20	200†	2000†	10^{-5}
1	53	64	75	77	69	1275
2	47	49	85	89	85	1302
3	67	41	63	73	55	1119
4	55	46	77	85	55	1438
Mean	56	50	75	81	66	1284
SD	8	10	9	7	14	131
SE	4	5	5	4	7	65

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 200 and 2000 µg/ml.

Table 121

UNSCHEDULED DNA SYNTHESIS ASSAY OF RESMETHRIN
(dpm/μg DNA)

Sample	Concentration of Compounds Tested: Resmethrin (μg/ml) 0*	100†	200†	400†	800†	1600†	4NQO (M) 10^{-5}
1	107	102	175	86	89	118	1832
2	85	263	78	150	97	91	1983
3	136	84	61	121	97	94	1800
4	188	112	211	166	121	81	1904
5	78	83	111	93	86	90	1870
6	76	60	160	90	114	85	1645
Mean	112	117	133	109	101	93	1839
SD	44	74	59	26	14	13	114
SE	18	30	24	11	6	5	47

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at all concentrations.

Table 122

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF RESMETHRIN
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	Resmethrin (µg/ml)					DMN (M)
	0*	4	40	400†	4000†	5×10^{-2}
1	99	112	105	89	105	368
2	121	103	102	96	107	397
3	120	78	115	93	114	387
4	141	85	105	107	125	381
Mean	120	95	107	96	113	383
SD	17	16	6	8	9	12
SE	9	8	3	4	5	6

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 400 and 4000 µg/ml.

Table 123

UNSCHEDULED DNA SYNTHESIS ASSAY OF RESMETHRIN WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Resmethrin (µg/ml)						DMN (M)
Sample	0*	250†	500†	1000†	2000†	4000†	5 x 10^{-2}
1	97	62	65	66	33	76	381
2	88	60	66	55	75	68	297
3	46	59	46	--‡	50	52	298
4	90	55	52	68	56	74	332
5	105	58	45	73	64	81	321
6	71	56	56	44	53	48	313
Mean	83	58	55	61	55	66	324
SD	21	2	9	12	14	13	31
SE	9	1	4	5	6	5	13

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at all concentrations.

‡Sample lost.

Table 124

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF SUMITHRIN
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Sumithrin (µg/ml) 0*	2	20	200†	2000†	4NQO (M) 10^{-5}
1	53	51	80	76	96	1275
2	47	59	93	85	54	1302
3	67	57	82	86	84	1119
4	55	62	96	100	98	1438
Mean	56	57	88	87	83	1284
SD	8	5	8	10	20	131
SE	4	2	4	5	10	65

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 200 and 2000 µg/ml.

Table 125

UNSCHEDULED DNA SYNTHESIS ASSAY OF SUMITHRIN
(dpm/µg DNA)

Sample	Concentration of Compounds Tested: Sumithrin (µg/ml) 0*	15	44	133†	400†	1200†	4NQO (M) 10^{-5}
1	65	104	92	102	91	90	1838
2	54	105	99	109	125	79	1776
3	97	128	133	96	82	107	1814
4	77	101	83	91	89	102	1681
5	81	97	87	130	79	131	816
6	63	84	100	93	94	129	1913
Mean	73	103	99	104	93	107	1639
SD	15	14	18	15	16	21	411
SE	6	6	7	6	7	8	168

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 133, 400, and 1200 µg/ml.

Table 126

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF SUMITHRIN
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested					
	Sumithrin (µg/ml)					DMN (M)
Sample	0*	4	40†	400†	4000†	5×10^{-2}
1	99	88	87	97	114	368
2	121	97	93	106	110	397
3	120	110	89	105	131	387
4	141	115	120	138	119	381
Mean	120	102	97	112	119	383
SD	17	12	16	18	9	12
SE	9	6	8	9	5	6

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 40, 400 and 4000 µg/ml.

Table 127

UNSCHEDULED DNA SYNTHESIS ASSAY OF SUMITHRIN WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Concentration of Compounds Tested						
	Sumithrin (μg/ml)						DMN (M)
Sample	0*	250†	500†	1000†	2000†	4000†	5×10^{-2}
1	97	68	51	75	68	60	381
2	88	64	59	51	65	51	297
3	46	69	69	52	70	66	298
4	90	96	55	56	83	51	332
5	105	54	47	65	59	49	321
6	71	65	46	84	60	63	313
Mean	83	70	54	64	68	57	324
SD	21	14	9	14	9	7	31
SE	9	6	4	6	4	3	13

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at all concentrations.

Table 128

FIRST PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF ZINEB
(dpm/µg DNA)

	Concentration of Compounds Tested					
	Zineb (µg/ml)					4NQO (M)
Sample	0*	2†	20†	200†	2000†	10^{-5}
1	53	13	3	1	1	1275
2	47	5	4	2	2	1302
3	67	2	1	4	1	1119
4	55	2	2	2	2	1438
Mean	56	5	2.5	2	1.5	1284
SD	8	5	1.2	1.2	0.6	131
SE	4	2.5	0.6	0.6	0.3	65

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at all concentrations.

Table 129

SECOND PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF ZINEB
(dpm/µg DNA)

Sample	Concentration of Compounds Tested					
	Zineb (µg/ml)					4NQO (M)
	0*	0.016	0.08	0.4	2.0	10^{-5}
1	107	156	94	112	95	1832
2	136	117	102	92	73	1983
3	85	156	106	77	90	1800
4	78	168	60	98	113	1904
Mean	102	149	91	95	93	1879
SD	26	22	21	15	16	81
SE	13	11	10	7	8	41

*Negative control and compound solvent, 0.5% DMSO.

Table 130

UNSCHEDULED DNA SYNTHESIS ASSAY OF ZINEB
(dpm/µg DNA)

Sample	Concentration of Compounds Tested						
	Zineb (µg/ml)						4NQO (M)
	0*	0.05	0.19	0.75	3.0	12†	10^{-5}
1	65	64	63	89	77	19	1838
2	54	64	63	68	84	24	1776
3	97	46	80	68	156	23	1814
4	77	70	95	79	87	20	1681
5	81	74	80	93	94	19	816
6	63	98	52	109	82	14	1913
Mean	73	69	72	84	97	20	1639
SD	15	17	16	16	30	4	411
SE	6	7	6	7	12	2	168

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at 12 µg/ml.

Table 131

PRELIMINARY UNSCHEDULED DNA SYNTHESIS ASSAY OF ZINEB
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested					
	Zineb (µg/ml)					DMN (M)
Sample	0*	2	20	200†	2000†	5×10^{-2}
1	99	125	91	11	0	368
2	121	120	102	11	0	397
3	120	--‡	128	7	2	387
4	141	104	127	9	2	381
Mean	120	116	112	10	1	383
SD	17	11	18	2	1	12
SE	9	5	9	1	0.5	6

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 200 and 2000 µg/ml.

‡Sample lost.

Table 132

UNSCHEDULED DNA SYNTHESIS ASSAY OF ZINEB WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested						
	Zineb (µg/ml)						DMN (M)
	0*	12.5	25	50	100†	200†	5 x 10^{-2}
1	41	45	49	52	102	10	331
2	55	52	50	51	62	19	355
3	58	73	47	65	122	14	349
4	50	49	61	48	106	16	370
5	64	55	55	42	87	25	340
6	92	60	72	43	102	24	409
Mean	60	56	56	50	97	18	359
SD	17	10	9	8	20	6	28
SE	7	4	4	3	8	2	11

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 100 and 200 µg/ml.

Table 133

SECOND UNSCHEDULED DNA SYNTHESIS ASSAY OF ZINEB
WITH METABOLIC ACTIVATION
(dpm/µg DNA)

Sample	Concentration of Compounds Tested Zineb (µg/ml) 0*	51†	71.4†	100†	140†	196†	DMN (M) 5 x 10^{-2}
1	58	65	73	67	14	--‡	315
2	58	62	62	86	27	7	314
3	75	68	104	--‡	22	7	386
4	58	62	70	76	12	5	372
5	70	61	79	75	28	--‡	317
6	70	86	93	89	13	--‡	279
Mean	65	67	80	79	19	6	330
SD	8	9	16	9	7	1	40
SE	3	4	6	4	3	1	16

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at all concentrations.

‡Sample lost.

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